Haya: The Saudi Journal of Life Sciences (SJLS)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2415-623X (Print)
ISSN 2415-6221 (Online)

Bio-Ethanol Derived from Less Edible Fruit Cultivars of Coorg District (Karnataka) and Parametric Analysis Using IC Engine Fuelled with Bio-Ethanol-Diesel Blends

Thouseef Ahamad MY[1], Panduranga Murthy G[2*], Rajesh Kumar[3], Manjunatha R[4], Leelaja BC[5]
[1,2]Department of Applied Sciences & Mechanical Engineering, Maharaja Institute of Technology, Thandavapura, Nanjanagud Tq, Mysuru district, Karnataka, India (Visvesvaraya Technological University, Belagavi, Karnataka).
[3]University Department of Chemistry, Ranchi University, Morabadi, Ranchi, (Jharkhand), India.
[4,5]Bhoomigeetha Institute of Research & Development (BIRD), B. H. Road, Tumkur, Karnataka, India.

Original Research Article

***Corresponding author**
Panduranga Murthy G

Article History
Received: 18.01.2018
Accepted: 25.01.2018
Published: 30.01.2018

DOI:
10.21276/haya.2018.3.1.15

Abstract: The sustainable production of ethanol from implausible bio-resources like, less or non-edible fruit samples are focused in the current investigation. Bio-ethanol is a fuel ethanol (ethyl alcohol), the same type of alcohol found in alcoholic beverages. It is most often used as a motor fuel, mainly as a bio-fuel additive for gasoline. Therefore, an attempt has been made to obtain a fuel grade ethanol using fruit samples like, Passion fruit (*Passiflora edulis,* L) and Cashew Apple (*Anacardium occidentale,* L.) by fermenting them with the help of flocculating yeast (*Saccharomyces cerevisiae,* L*)*. The outcome of the experimentation facilitated to obtain 48% alcohol after finishing point of distillation processes; it can be can envisioned that, an optimized concentration of ethanol has been obtained on ensuing to standardization of the protocol. This bio-ethanol has been further subjected for physico-chemical characterization and found that, it can be one of the most promising types of bio-fuels that can be explored from the fruit resources. Hence, in the studies, the technological attributes of flocculated Yeast (*Saccharomyces cereviseae*) was employed to rationalize its use in alcoholic fermentation, thereby, the enhancement in the production of bio-ethanol from the selected fruit cultivars at significant level was achieved with a speed of about three to four folds greater than the yield reported in the earlier studies. Further, the ethanol samples were evaluated for Gas chromatography; the yield of ethanol, sample-4 (Passion fruit juice) gave 100% purity of ethanol as compared to standard. The bio-ethanol obtained as a result of fermentation was further subjected for the evaluation of Engine performance using single cylinder IC Engine with standard specifications. The analysis revealed a most significant performance with the variable blends of Bio-ethanol and Petroleum diesel (B10-15). Besides, a remarkable reduction in the release of hazardous gases (COX, NOX) with the blends of bio-ethanol (B10-15) was noticed. In due course, the bio-ethanol produced from non-edible fruit samples was found to be of high quality, which can be recommended as an alternative promising bio-fuel in the present engine systems as it meets the ASTM standards with regards to both performance and emission parameters in particular, viscosity, flash point, cetane number, calorific value and metal content etc. Subsequently, the designed protocol has been materialized through the assessment of various fuel efficiency parameters and found that, the bio-ethanol can be considered as cost effective, much cleaner, eco-friendly and does not yield any toxic residues or gases to the environment. Hence, a good quality of bio-ethanol production from less/non-edible fruit samples has been justified ultimately through the parametric analysis and the same can be exercised further in the industries unambiguously by Automobiles and transportation.

Keywords: Bio-ethanol, Less Edible Fruit cultivars, Cashew apple, fashion fruit, Yeast, Fermentation, IC Engine, performance, emission parameters.

INTRODUCTION

The world scenario on the energy requirements are mainly based on fossil fuels. However, these fuel reserves are finite and their exploitation has created serious environmental problems. As a consequence, the demand for alternative renewable sources has been intensified *visa-vis*; the rising demand for energy and raw materials [1]. The possibility and production of green energy (environment friendly) from bio-waste material has played an important role in recent days due to the depletion of non-renewable energy resources [2, 3]. Hence, the biological sources are emerging as most preferred sources that are potential to meet the current challenges of sustainability. An increasing number of developed and rapidly developing nations can see bio-fuels as a key progressive factor in reducing the reliance on foreign oil, subsequently; green fuel also lowering emissions of green house gases (GHG), mainly carbon dioxide (CO_2) and methane (CH_4), and meeting rural development goals [4, 5, 2, 1, 6].

The recent statistics is overwhelmed by difficulties like decrease in the petroleum fuels extracted from fossil resources, rapid rise in green house gas emissions contributing to global warming, and the lack of potential raw materials to meet the ever-increasing energy demands. In this context, many attempts were made to reduce the negative impact of these global concerns by focusing on alternative sources. The bio-ethanol produced from renewable resources like biomass components has created significant interest. Through preliminary investigations, bio-ethanol is found to be most promising alternative fuel which is an alcohol found in alcoholic beverages. The alcohol based liquid fuel made from different bio-resources is of great interest and the bio-ethanol emerged as an important bio-fuel, having high calorific value in addition, it has the added advantage of being less polluting than most sources of energy that are in practice today [7, 8]. Besides, it is most often used as motor fuel mainly as bio-fuel in association with gasoline. Consequently, the ethanol, unlike petroleum, is claimed to be a form of renewable energy that can be produced also from agricultural crops such as sugar cane, potato, corn etc which are evidenced with previous reports and suggests that, previous natural substrates for ethanol production *via* saccharification have included sugarcane bagasse, wheat straw, corn, softwood, less edible fruits resources respectively [9-12].

Based on the basic hypothesis, the Fruit samples comprising of peels, flush & entire portions are found to excellent sources of cellulose which can be used for the production of ethanol *via* saccharification process followed by fermentation [13,14]. Besides, bio-fuel production from renewable sources is widely considered to be one of the most sustainable alternatives to reduce the burden on petroleum sourced fuels and a viable means for environmental and economic sustainability [15, 16, 3].

Coorg, commonly known as Kodagu is the smallest district in Karnataka (India). The region of Kodagu is configured with mountains and hillocks with rich biodiversity. Agriculture is the main developed sector in Kodagu and the main crops growing in the district are cardamom, pepper, coffee, cinchona, ginger & paddy respectively. In addition, the fruits such as limes, cashew apple, baddpulis, oranges, passion fruit, butter fruit, banana, pineapple, fig, gooseberry and sapota's are expansively grown. Now with the exception of orange, lime and banana cultivation no other fruit is grown economically. Approximately sixty five percent of Kodagu's geographical area is under tree cover and it is also one of the densely forested districts of India. The uniqueness of Kodagu region is expressly regarded as 'hotspot of biodiversity' approaching within the purview of Western Ghats as it has diverse kinds of vegetation. Since, the climate is considerably cool throughout the year; wine preparation is common business in most of the household. Many of them prepare wine in large quantity and sell it. The commonly prepared wines are of goose-berry, passion fruit, grape, pineapple, Cashew apple, lime, rice, betel, ginger, fig, banana etc.

In the similar perspective, the juice industries of certain fruits, such as passion fruit, grape, pineapple, cashew apple are grown in almost all local territory of Coorg districts; which generate large amounts of waste (peels, flush and seeds) from the crush of tons of fruit to obtain the juice. Thus, there is a great deal of less edible fruit samples especially, passion fruit and cashew apple fruit waste that could be processed for the production of ethanol [17].

However, utilization of these fruit materials in the production of bio-ethanol would be of great environmental and economic benefit as it could reduce the burden on conventional sources of energy and also get rid of the managerial issues of bio-wastes. Hence, the present study has been initiated to evaluate the juices extracted from less edible fruit cultivars explicitly, *passion fruit (Passiflora edulis*, L.) and Cashew apple (*Anacardium occidentale*, L.) for the production of Bio-ethanol by employing fermentation process using flocculating Yeast (*Saccharomyces cerevisiae*).

MATERIALS AND METHODS

The study was initiated with the baseline survey conducted at different biodiversity regions of Kodagu district (Karnataka). During the survey, interactions were made with local farmers and fruit growers followed by many household people who prepare wine and sellers through an approach of family enterprise. Further, the pre-arranged interaction was also extended with commercial wine vendors regarding the present scenario emphasizing on collection of fruit material, processing, preparation of wine and market value of these home-made wine varieties. The first hand information was gathered through semi-structured questionnaire.

Collection of Fruit Materials

The fruit materials; *Passiflora edulis* (Passion fruit) and *Anacardium occidentale* **(**Cashew Apple**)** used in this study were procured from the local fruit growers of Coorg district, Karnataka (INDIA) during the period; August, 2014 to October, 2015 both in pre and post-harvest seasons respectively. The plantation of both Passion fruit and Cashew Apple is located along the eastern part in the rural province of Kodagu district. A local-wild variety of cashew apples that did not show prominent cashew-nut attachment; these were selected. Similarly, yellow and purple coloured samples of Passion fruits were procured from the fruit orchards by the Farm Manager (Mr. Rauf). The details of both fruit cultivars are briefed hereunder.

Passion fruit

Passion Fruits (*Passiflora edulis*, L) are commonly grown in cold regions. Basically, passion fruits are abundantly grown in South American countries. In India, these fruits are extensively grown in different parts of Coorg and Malnad area of Karnataka. These fruits are also grown in Kodaikanal region of Tamil Nadu and Wayanad region of Kerala. Visually, the passion fruits in this area are appears to be as yellow and purple color which are most commonly available in the Coorg region (Fig-A).

Cashew apple fruit

Cashew apples grown from the cashew tree (*Anacardium occidentale* L.), which is basically a native exotic plant in Brazil. The largest cashew growing regions are Brazil, India, Nigeria, Tanzania, Indonesia and Vietnam. I India, these fruits are grown expansively in specific regions of Coorg district followed by Malnad (mountain regions) area of Karnataka. The cashew apples are a false fruits and are physically described as small, hard, pear-shaped green fruit and when ripened turn red, yellow or orange (Fig - B).

Processing Fruit cultivars (substrates)

The fruit samples of *Passiflora edulis* (Passion fruit) and *Anacardium occidentale* (Cashew Apple) were obtained from local growers of Coorg district (Karnataka) as mentioned above. The Passion fruits were washed under running tap water and are peeled off and are subjected for squashing using suitable Mixer. The squashed fruits were then filtered using white cloth. The filtered squash was transferred to plastic barrel and the sugar syrup is added to this; the desired microbial strain, yeast was added along with potassium-meta-bi-sulphate to avoid undesired growth microbes in the fermentation processes. In the other barrel to the same quantity of contents instead of yeast wheat is added. The barrels are kept at room temperature for about 14 days.

In case of Cashew Apples; the healthy uniform sized fruits were washed twice with distilled water (to remove surface dirt particles present if any) before being processed for use. The fruit materials were then subjected for drying in a hot air oven at 65˚C for 24 hours and powdered using a suitable grinder. The fruit for about 50g of each rind was weighed and utilized as the substrate [18, 19].

Extraction of Juice from Fruit cultivars

The extraction of juice from both Passion Fruit Juice (PFJ) and Cashew Apple Juice (CAJ) was done at the Applied Science Laboratory, MITT in association with Bhoomigeetha Institute of Research & development (BIRD), Mysore. The whole fruits were washed individually and cut into medium pieces. The fruit pieces were then compressed in a fruit processor with a separate pulp collector. Whilst extracting, the PFJ and CAJ were kept on ice cold followed by frozen condition and further was subjected for storage at -20°C for subsequent use [20].

Physico-Chemical Characterization of Juice extracted from Fruit cultivars

The juices extracted from both fruit cultivars were then subjected for physico-chemical parameters all through the fermentation and the generated data was represented systematically in the form of tables respectively. Besides, the parameters such as reducing sugar, pH, Effect of specific gravity, Titration, Concentration of ethanol are continuously monitored during the fermentation.

Microorganism and Culture media

The yeast, *Saccharomyces cerevisiae* was procured from authorized sources, (Azyme Technologies, Bengaluru). The yeast was then cultured on Yeast extract Peptone Dextrose (YPD) at 30˚C. The cultures were stored at 4˚C and sub-cultured for every 30 days and the same was subjected for flocculation in the order to elicit their activation at the time of experimentation.

Preparation of PFJ and CAJ for fermentation

The fruit juice; PFJ and CAJ were prepared afresh prior to each fermentation course by pre-treatment, centrifugation and sterilization. The pre-treatment involved the addition of 1% (w/v) gelatin powder to the raw PFJ and CAJ were maintained at 4°C for 24hrs. This was followed by centrifugation at 3500 rpm for 20min, addition of 2.5 g/L of ammonium sulphate and sterilization at 121°C for 15min. Later, the PFJ and CAJ were sterilized in conjunction with the fermentation vessel [21].

Fermentation

Preparation of Yeast Inocula

In the experimentation, the yeast inoculums were prepared in sterilized yeast peptone dextrose (YPD) liquid broth. The composition of YPD in g/L was 10g yeast extract powder, 20g peptone powder and 20g D-glucose. The culture conditions were as follows: (i) volume = 200mL, (ii) temperature = 30°C, (iii) agitation = 150 rpm and (iv) duration = 18 hrs. The yeast was activated by extracting the glycerol and re-suspending of the pellet in 1mLof YPD broth and this suspension was added to 50mL of YPD liquid broth. The cell count was increased by sub-culturing followed by flocculation at 10% every 18 hrs from 50mL to

200mL (32). After cultivation, the cell viability was determined and 10% of the initial fermentation volume was used as a starter culture [22, 18, 23].

Saccharification and its knocking action

The process, Saccharification is the critical step for bio-ethanol production where; complex carbohydrates are converted to simple monomers. Compared to acid hydrolysis, enzymatic hydrolysis requires less energy and with mild environment conditions. The processed fruit materials were added to a broth containing yeast extract (5g/l) and peptone (10g/l). The media was autoclaved at 121^{o}C and 15 psi pressure for 20 min and yeast was inoculated under aseptic conditions. The flasks were then incubated at room temperature for a period of 144 hrs.

Subsequent to saccharification, the cultures were autoclaved and filtered using what man No. 1 filter paper. The filtrate was then transferred into 250ml Erlenmeyer flasks, made airtight with cork and autoclaved through unit operation. The flasks were then aseptically inoculated with 15ml over night grown yeast (S. *cerevisiae*) and incubated at room temperature for 96hrs.

The fermentation of fruit Juice of both *Passiflora edulis* (Passion fruit) and *Anacardium occidentale* (Cashew Apple) was performed with a fermenter of standard specification; A BIOSTAT Bplus2L MO-O2 (Sartorius Stedim Systems GmBH, Germany) to facilitate the production of bio-ethanol. The operating conditions of the batch fermentations are summarized in the Table-2. The fermentations were performed in duplicate.

The ethanol produced was then determined by Gas Chromatography (Bipolar, 1250 mV, 10 Samp) technique using a NUCON Gas Chromatograph (5765 EPC) with a flame ionization detector per sec. The carrier gas used in the experimentation was Nitrogen.

Distillation

In the last part of experimentation, the batch distillation method was adopted. The distillation unit consisting of three components: a re-boiler, condenser pipe and a distillate or receiving flask. The filtered samples was transferred into the re-boiler and heated to boil respectively. The vapors started to rise into the still head and passed through condenser pipe. The continuous circulation of cold water around the condenser pipe assisted in cooling the alcohol rich vapors back to liquid state. The condensed liquid enters the still receiver and then collected in the distillate. The distillate was then tested for its alcohol content using Syke’s hydrometer. Simultaneously, the temperature of the distillate was also measured. The alcohol content and the temperature were used to find the percentage of ethyl alcohol (EtOH) of the distillate, referring to the distillation chart was recorded. It was found that the percentage of ethyl alcohol was in the range of 6 % - 8 % depending upon the various ranges of different parameters.

Reducing Sugar assay

The ethanol fermentation is a biological process in which; sugars are converted by the influence of microorganisms used in the experimentation to produce ethanol and CO_2. The microorganism most commonly used in fermentation process is the yeast and among the yeasts, *Saccharomyces cerevisiae* is the preferred choice for ethanol fermentation [24].

Reducing sugars assay was carried out according to the Di-nitro-salicylic (DNS) method. The un-inoculated media was used as control for the assay. The optical densities of the samples were measured against the blank at 540nm.The glucose concentration was then calculated using standard glucose curve.

Gas Chromatography (GC) Analysis

The content of ethanol concentration was measured using this technique for the different samples. The analysis performed by a gas chromatograph is called Gas chromatography which analyzes the compound that can be vaporized without decomposition and GC may help in identifying a compound with purity status. In the experiment, the GC technique was adopted to analyze the actual concentration of ethanol and the purity of the given sample was also analyzed.

Generally chromatographic data has been presented in the graphical format comprising of detector response (y-axis) against retention time (x-axis), which is called a chromatogram. This provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from the column at different times. The retention time can be used to identify analytes if the method conditions are constant. In addition, the pattern of peaks will be constant for a sample under constant conditions and can identify complex mixtures of analytes. However, in most modern applications, the GC is connected to a mass spectrometer or similar detector that is capable of identifying the analytes represented by the peaks.

Experimental setup for IC (Internal Combustion) Engine Test Rig

The experimental test rig was developed keeping the necessary configurations of the IC Engine test rig using single cylinder, four stroke, air cooled and IC diesel engine which developed a maximum power output of 4.5 kW at constant speed of 1600 rpm (Table-12 & Fig-1).The schematic diagram of the experimental setup along with mechanism of action during fuel supply was also analyzed (Fig-2 & 3).The performance and emission parameters were carried-out with variable Bio-ethanol-Petroleum fuel blend ratios respectively based on the standard protocols.

RESULTS AND DISCUSSION

The fruit cultivars; Cashew apple and Passion fruit were daily monitored (Fig-A & B). During the course of fermentation; various parameters such as pH, specific gravity, alcohol concentration and titration was determined (Table-1, 2 & 3). The results show that, the fermentation is considerably fast in yeast compared to wheat fermentation described in the earlier reports [25, 23, 19]. The result also shown that; the production of ethanol is also significantly more in case of fermentation using flocculating yeast. This is in accordance with the earlier reports made by Flávia Cristina dos Santos Lima *et al*., [26] and Evanie Devi Deenanath *et al* ., [27].

Juice Preparation for Passion fruit & Cashew Apple samples

The juice yield was found to be most significant in both Passion fruit and Cashew Apple fruit samples. The measure of 20 kg PFJ (yielded 9.85 L) and CAJ (yielded 5.6L) yielded considerable amount of juice during extraction process. Prior to fermentation process, both PFJ and CAJ were thawed, centrifuged and pre-treated with gelatin powder to remove some secondary metabolites. The preparation of PFJ and CAJ was essential as the clear juice with a minimal to zero suspended solids which will allow the yeast to easily assimilate the sugars for growth and the production of ethanol (Table-4 & 5).

Physico-Chemical Characterization

The Juice extracted from *Passiflora edulis* and *Anacardium accidentale* all through the fermentation process was subjected for physico-chemical characterization executed as per the standard protocols. The data has been represented in the tables respectively (Table-1 & 2).

Evaluation of pH and Moisture

The variation in pH was seen during execution of the experiment. The decreasing trend of pH was observed at initial stage, then it was attained a minimal stage and then achieved increased approach. The initial pH for passion fruit was 5.7 then; it decreased to 4.9 and again it increased to 6.0. Subsequently, in passion fruit; it was 5.5 pH initially then decreased to 5.0 pH and increased in the range of 5.2-6.0pH respectively. Similarly, for Cashew apple, the initial pH was 3.8then increased to 5.8pH and the final range of pH was found to be 6.0. The Moisture content (%) of Passion fruit 13.45±0.04 and Cashew apple 9.25±0.01 was noticed after pre-treatment of the substrates was represented in the Table-3 [28-31, 9].

Evaluation on reducing sugar

The Reducing sugar in terms of percentage was analyzed and the result reveals; 0.74% in Passion fruit samples and 0.58% was noticed in Cashew apple fruit sample (Table-3). The yeast can grow both on simple sugars, such as glucose and on the disaccharide sucrose. Furthermore, the availability of a robust genetic transformation system of *S. cerevisiae* along with a long history of this microorganism in industrial fermentation processes makes it most desired microorganisms for ethanol production [21, 17, 32]. The *S. cerevisiae* has high resistance to ethanol, consumes significant amounts of substrate in adverse conditions and shows high resistance to inhibitors present in the medium [33, 34, 27].

Effect of Specific gravity

When the fermentation starts; the specific gravity decreases. The specific gravity of passion fruit reached 0.998 at 52 hours, whereas, specific gravity of cashew apple was reached to 0.887 respectively (Table-4 & 5). After 14 days it reached a constant value indicating the end of fermentation [22, 20, 27].

Assessment on Titration

As the days increased fluctuating trend was seen in titration. The titratable acidity for passion fruit was 4.42g/L tartaric acid at the starting and reached 9.96 on last day. In case of Cashew apple it was 3.23 at the start and reached 8.89g/l tartaric acid on the last day [35, 12].

Concentration of ethanol

The ethanol content in the distillate was measured. The concentration of bio-ethanol in passion fruit sample was 11.4%, whereas; the concentration of ethanol in cashew apple was 8.3% (Table-4 & 5). This value is higher than the report published by [18, 19, 35, 27].

Gas Chromatography (GC) Analysis

The function of the stationary phase in the column has separated ethanol, causing each one to exit the column at a different time (retention time). The other parameter that was used to alter the order or time of retention is the carrier gas flow rate, column length and the temperature.

The purity of the carrier gas was also frequently determined by the detector, though the level of sensitivity needed can also play a significant role. The spectrum of peaks for different samples representing the analytes present in the given samples eluting from the column at different times (Graph-3-8). The purity of 100% bio-ethanol was noticed in Passion Fruit samples executed with increased days of fermentation using flocculating yeast cultures. The result of the given samples comprising of sample 1- 8.7 ethanol, sample 2 -15.8 ethanol, sample 3 - 11.6 ethanol respectively (Table-6-10 & Graph-1-5). Among all the samples, sample 4 achieved 5.3% ethanol which gives 100% purity as compared to standard ethanol (Table-9 and Graph 4 & 6). The similar results were also obtained by previous researchers [36-39, 12].

Fig.A: Passion Fruit B: Cashew Apple

Table-1: Physico-chemical characterization of Juice extracted from *Passiflora edulis* all through the Fermentation process

Time (Days)	Yeast count *S. cerevisiae* (Log-CFU/ml)	Total soluble solids (°Brix)	pH	Alcohol (%-v/v)	Titratable Acidity as Citric Acid (%)	Fermentation Temp (°C)	Sugar Content (g/l)	Specific Gravity
01	03	14	3.1	0.0	0.68	20	92	1.064
02	04	11	3.2	3.6	0.72	20	85	1.052
03	05	08	3.4	4.8	0.79	22	79	1.048
04	06	06	3.5	5.4	0.86	24	71	1.042
05	07	05	3.8	6.5	0.89	25	68	1.037
06	09	04	3.9	8.7	0.92	26	59	1.033
07	10	02	4.2	9.5	0.95	28	52	1.028
08	12	1.5	4.5	10.7	0.98	30	48	1.024
09	13	1.4	5.2	12.4	1.15	32	41	1.018
10	14	0.8	5.8	12.9	1.21	34	38	1.014
11	15	0.5	6.0	13.5	1.28	35	34	1.008
12	18	0.3	6.5	15.8	1.45	36	31	1.006
13	20	0.2	6.6	18.6	1.56	38	28	1.005
14	24	0.07	6.8	19.5	1.66	40	19	1.002

Table-2: Physico-chemical characterization of Juice extracted from *Anacardium accidentale* all through the Fermentation process

Time (Days)	Yeast count *S. cerevisiae* (Log-CFU/ml)	Total soluble solids (°Brix)	pH	Alcohol (%-v/v)	Titratable Acidity as Citric Acid (%)	Fermentation Temp (°C)	Sugar Content (g/l)	Specific Gravity
01	03	20	3.1	0.0	0.72	20	88	0.996
02	04	19	3.2	3.2	0.74	20	80	0.952
03	05	18	3.4	4.5	0.78	22	75	0.946
04	06	16	3.5	5.6	0.80	24	68	0.938
05	07	13	3.8	6.7	0.82	25	62	0.922
06	09	11	3.9	8.4	0.86	26	56	0.908
07	10	08	4.2	9.6	0.92	28	50	0.894
08	12	6.5	4.5	10.4	0.96	30	46	0.882
09	13	4.4	5.2	11.6	1.00	32	44	0.865
10	14	3.2	5.8	11.9	1.14	34	42	0.860
11	15	2.5	6.0	12.2	1.24	35	38	0.846
12	18	2.1	6.5	12.5	1.42	36	36	0.828
13	20	1.8	6.6	12.8	1.49	38	35	0.798
14	24	1.6	6.8	13.6	1.54	40	34	0.786

Table-3: Showing Physico-chemical characteristic features in Passion fruit and Cashew apple after pre-treatment

SL. No.	Parameters analyzed	Passion fruit Juice(PFJ)	Cashew Apple Juice ICAJ)
1.	pH	5.2—6.0	5.8-6.0
2.	Moisture (%)	13.45±0.04	9.25±0.01
3.	Soluble Solids(^{0}Brix)	0.00±0.00	0.004±0.00
4.	Reducing Sugar (%)	0.74±0.02	0.58±0.08

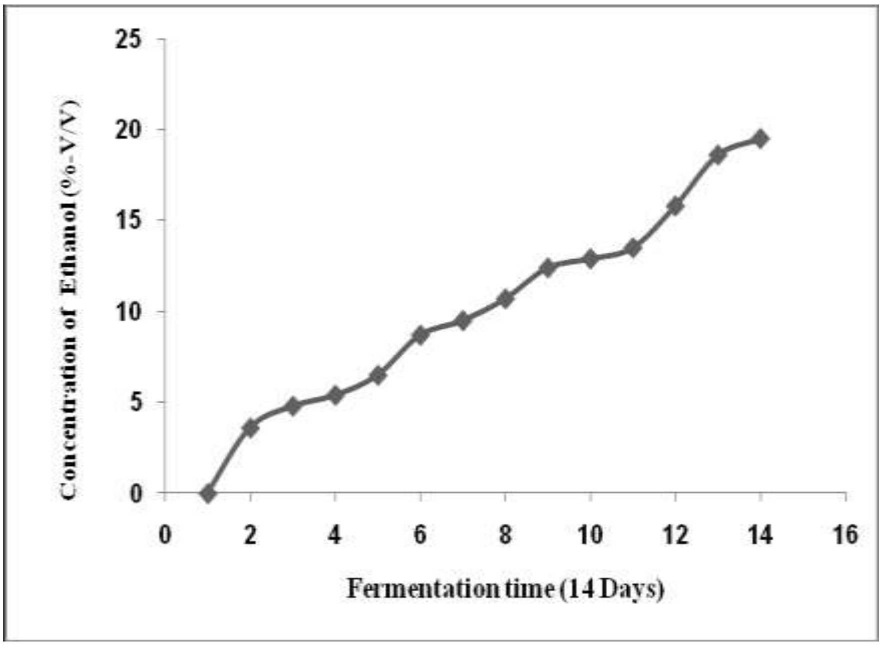
Table-4: Showing different parameters and respective yields of Ethanol for Passion Fruit samples

SL. No.	Wine(Yeast)	pH	Specific Gravity	Ethanol Conc.%
01	Passion fruit	6.0	0.998	15.2
02	Passion fruit	5.2	0.890	11.4
03	Passion Fruit	5.9	0.833	8.2

Table-5: Showing different parameters and respective yields of Ethanol for Cashew Apple Fruit samples

SL. No.	Wine(Yeast)	pH	Specific Gravity	Ethanol Conc. %
01	Cashew Apple	6.0	0.887	8.3
02	Cashew Apple	5.8	0.850	7.0
03	Cashew Apple	5.8	0.733	6.5

Graph-1: Showing Ethanol concentration (%) v/s Days of Fermentation in Passion fruit sample



Graph-2: Showing Ethanol concentration (%) v/s Days of Fermentation in Cashew apple fruit sample

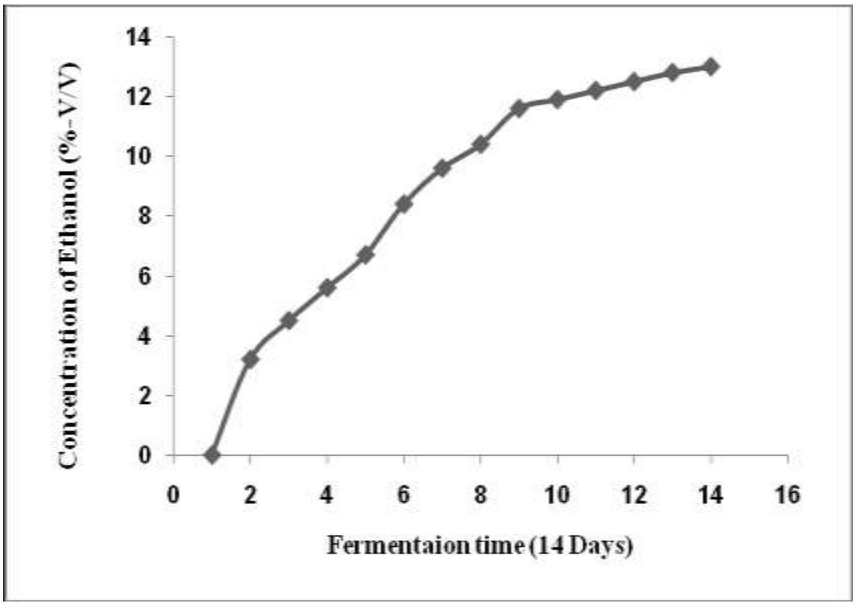


Table-6: Showing GC Analysis of Juice sample of *P. edulis* after fermentation using flocculated Yeast

SL.No.	Retention Time(Time)	Area(mV.s)	Area(%)
1.	2.350	87.450	86.80
2.	2.877	13.345	13.20
Total		100.79	100.0

Graph-3: GCA analysis of fruit juice sample of *Passiflora edulis* after fermentation using flocculated Yeast

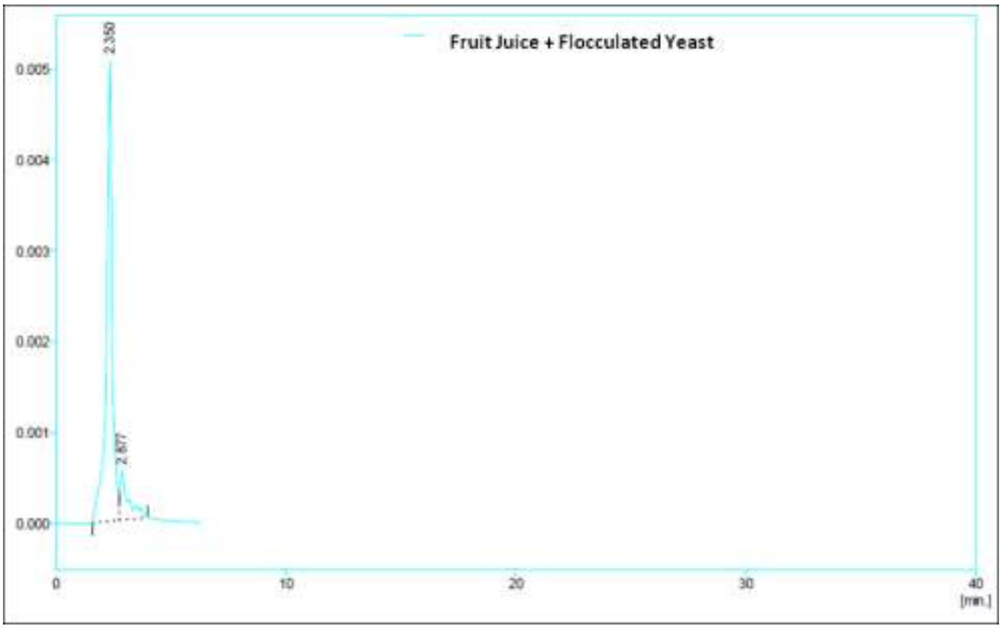


Table-7: Showing GC Analysis of fruit juice sample of *A. accidentale* after fermentation using Yeast

SL. No.	Retention Time (Time)	Area (mV.s)	Area(%)
1.	1.917	5.124	3.1
2.	2.353	158.534	96.9
Total		163.658	100.0

Graph-4: GC Analysis of fruit juice sample of *A. accidentale* after fermentation using Yeast

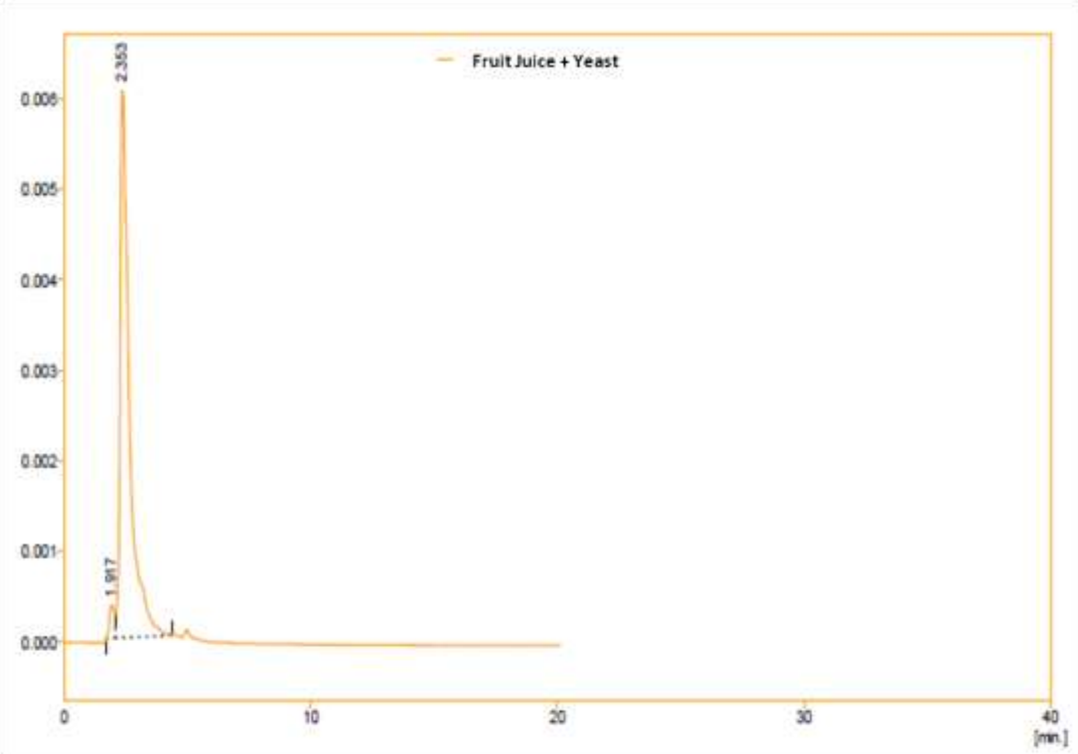


Table-8: Showing GC Analysis of fruit juice sample of *P. edulis* after fermentation using flocculated Yeast

SL. No.	Retention Time (Time)	Area (mV.s)	Area (%)
1.	1.917	2.890	2.4
2.	2.353	116.721	97.6
Total		119.611	100.0

Graph-5: GC analysis of fruit juice sample of *P. edulis* after fermentation using flocculated Yeast

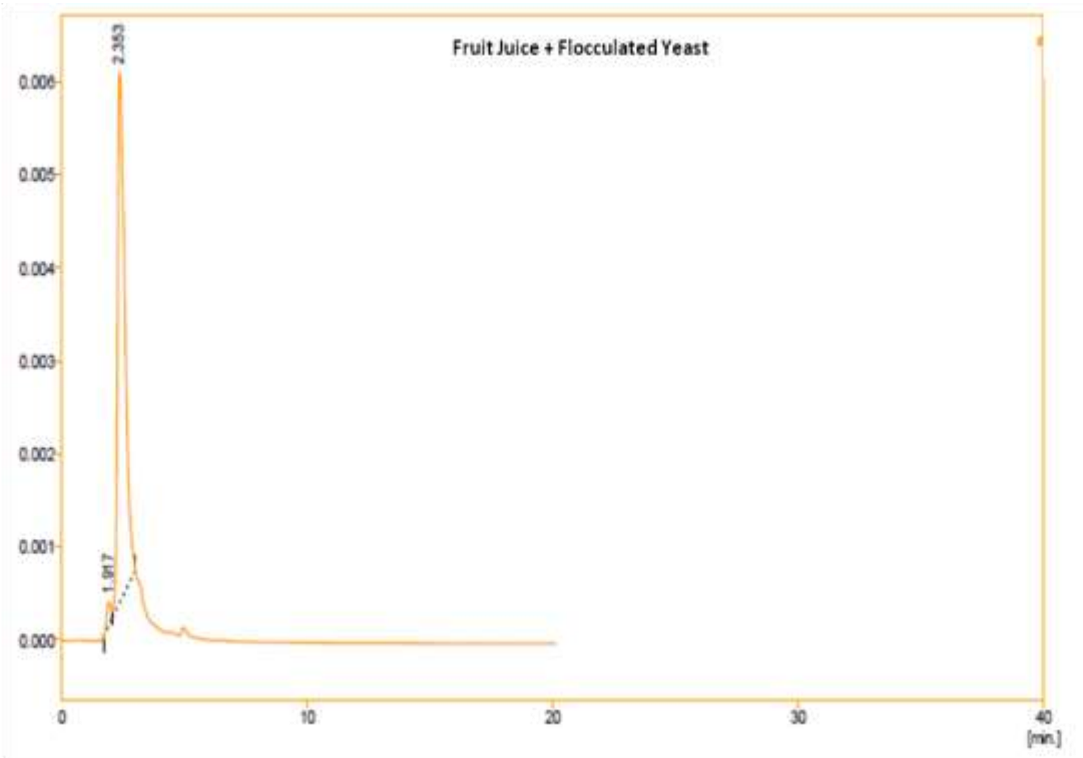


Table-9: Showing GC Aanalysis of fruit juice sample of *A. accidentale* after fermentation using flocculated Yeast with increased duration

SL. No.	Retention Time (Time)	Area (mV.s)	Area (%)
1.	2.553	53.367	100.0
Total		119.611	100.0

Graph-6: GC analysis of fruit juice sample of *A. accidentale* after fermentation usingflocculated Yeast with increased duration

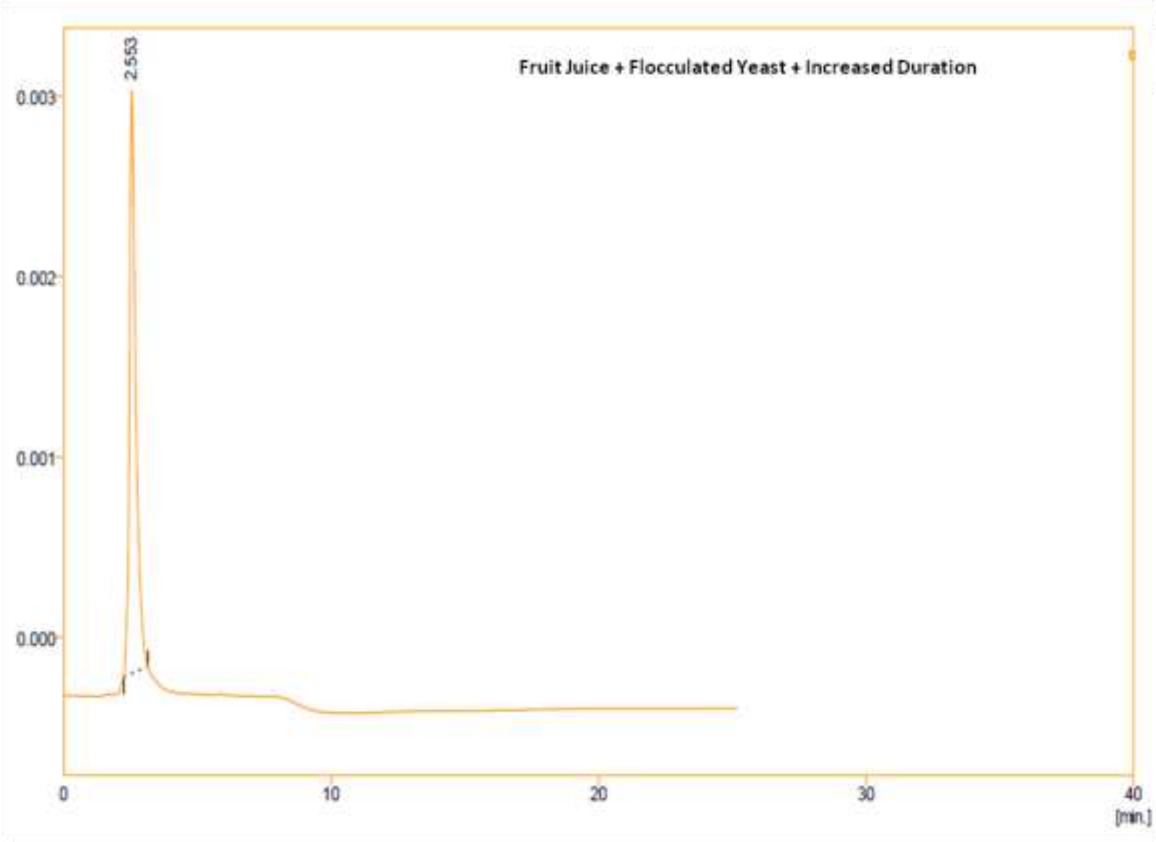
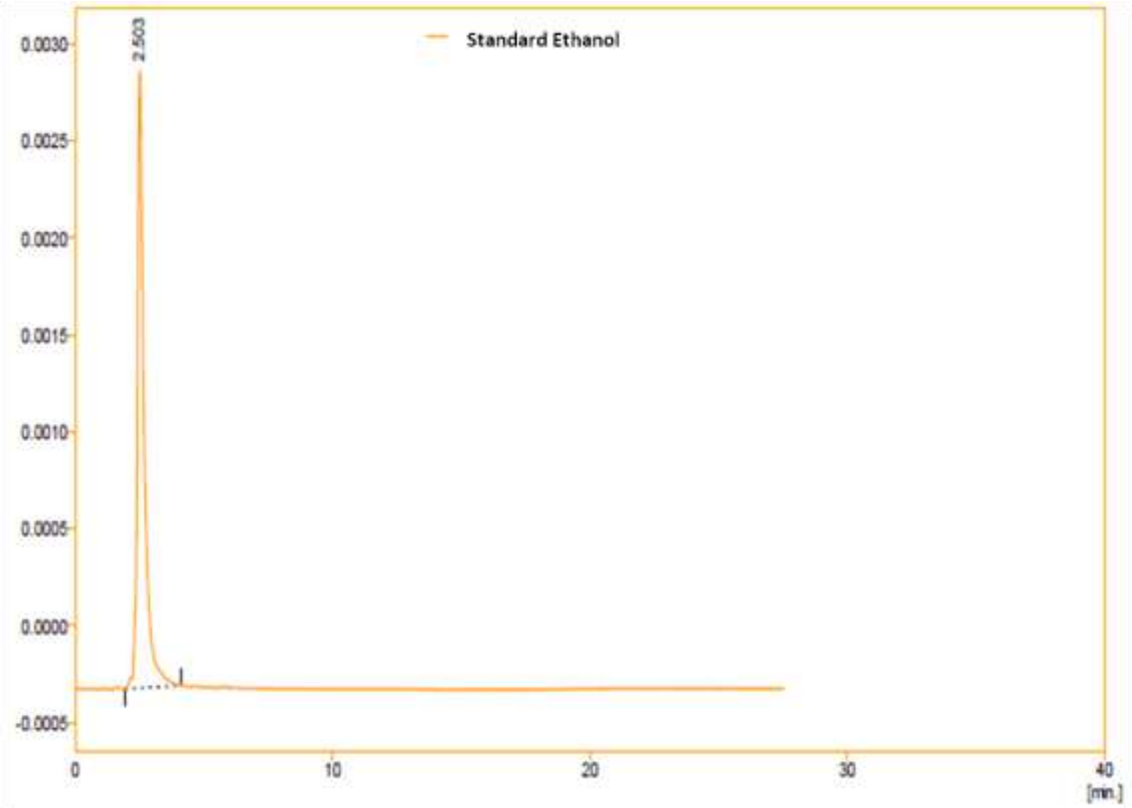


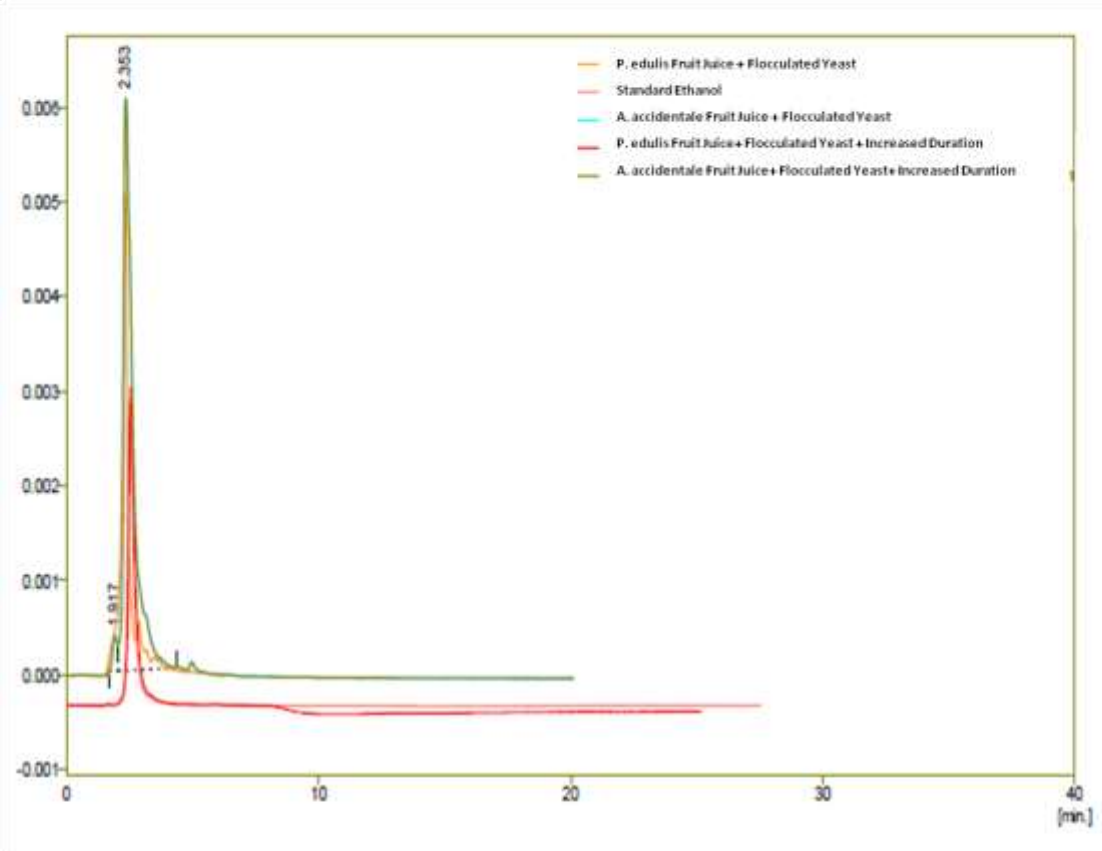
Table-10: Showing GC-MS analysis of Standard Ethanol

SL. No.	Retention Time (Time)	Area (mV.s)	Area (%)
1.	2.503	58.483	100.0
Total		58.483	100.0

Graph-7: GC Showing of Standard Ethanol



Graph-8: Showing GC Aanalysis of *Passiflora edulis* and *Anacardium accidentale* fruit samples comparing with Standard



Characteristic features of Bio-ethanol

The obtained Bio-ethanol from non-edible fruit samples was subjected for parametric analysis with respect to various properties as per the ASTM standards. In the evaluation, ethanol was found to be momentous with high octane number, low flame temperature, high density and high latent heat of vaporization. The ethanol also provides reduction in emission and increases the performance of CI engine, like specific fuel consumption, brake power, torque, volumetric and brake thermal efficiency (Table-11).

Table -11: Characteristic features of Bio-Ethanol, Petrol, and Diesel.

SL. No.	Properties	Unit	Bio-ethanol	Diesel oil
	Chemical Formula	---	CH_3CH_2OH	$C_{12}H_{23}$
1.	Density at 15^{O}C	Kg.m^{-3}	826	865
2.	Lower Calorific Value	MJ.kg^{-1}	32.8	45.6
3.	Octane Number VM	--	125	--
4.	Cetane Number	--	9	56
5.	Specific gravity	@ 15^{o}C	0.826	0.814
6.	Kinamatic Viscosity	@ 40^{O}C (cSt)	2.45	2.21
7.	Boiling Point	(^{O}C)	185-355	--
8.	Flash Point	(^{O}C)	36	30
9.	Calorific value	KJ/Kg	28440	46820
10.	Auto Ignition Temp.	^{o}C	372	240

Engine Efficiency Parameters

In the study, an internal combustion engine (ICE) was used to analyze the performance of and efficiency of Bio-ethanol derived from less edible fruit bio-resources. The IC engine is principally a heat engine where; the combustion of a fuel occurs with an oxidizer (usually air) in a combustion chamber that is an integral part of the working fluid flow circuit. In an internal combustion engine, the expansion of the high-temperature and high-pressure gases produced by combustion applies direct force to some component of the engine. The force was applied most typically to pistons, turbine blades, rotor or a nozzle. This force moves the component over a distance and transforming chemical energy into useful mechanical energy (Table-12 and Fig-1A & B, 2 & 3).

In the IC Engine system, *the top dead center* (TDC) of a piston is the position where it is nearest to the valves; *bottom dead center* (BDC) is the opposite position where it is furthest from them. A stroke is the movement of a piston from TDC to BDC or *vice versa*, together with the associated process. While an engine is in operation, the crank-shaft rotates on continuous mode virtually at a constant speed. In a 4-stroke ICE, each piston experiences 2-strokes per crankshaft revolution in the following approach.

Intake, **Induction** or **Suction**

The intake valves are open as a result of the cam lobe pressing down on the valve stem. The piston moves downward increasing the volume of the combustion chamber and allowing air to enter in the case of a CI engine or an air fuel mix in the case of CI engines which do not use direct injection. The air or air-fuel mixture is called the *charge* in any case.

Compression

In this stroke, both valves were closed and the piston moves upward reducing the combustion chamber volume which reaches its minimum when the piston was at TDC. The piston performs work on the charge as it was being compressed; as a result its pressure, temperature and density increase; an approximation to this behavior was provided by the ideal gas law. Just before the piston reaches TDC, ignition begins. In the case of a CI engine, the spark plug receives a high voltage pulse that generates the spark which gives it its name and ignites the charge. The fuel injector quickly injects fuel into the combustion chamber as a spray; the fuel ignites due to the high temperature.

Power or **working stroke**

The pressure of the combustion gases pushes the piston downward, generating more work than it required to compress the charge. Complementary to the compression stroke, the combustion gases expand and as a result their temperature, pressure and density decreases. When the piston was near to BDC the exhaust valve opens. The combustion gas expands irreversibly due to the left-over pressure in excess of back pressure, the gauge pressure on the exhaust port; this is called the *blow down*.

Exhaust

The exhaust valve remains open while the piston moves upward expelling the combustion gases. For naturally aspirated engines a small part of the combustion gases may remain in the cylinder during normal operation because, the piston does not close the combustion chamber completely; these gases dissolve in the next charge. At the end of this stroke, the exhaust valve closes, the intake valve opens, and the sequence repeats in the next cycle. The intake valve may open before the exhaust valve closes to allow better scavenging (Fig-3).

Table-12: Technical specifications of CI Engine

Sl. No.	Components	Specification
1.	No. of cylinder	1
2.	Type of injection	Direct
3.	Rated power at 1500 rpm (kW)	4.41
4.	Bore (mm)	87.5
5.	Stroke (mm)	110
6.	Compression ratio	17.5
7.	Method of cooling	Air cooled with radial fan
8.	Displacement volume, liters	0.662
9.	Fuel injection timing BTDC	23^{o}C
10.	Number of injector nozzle holes	3

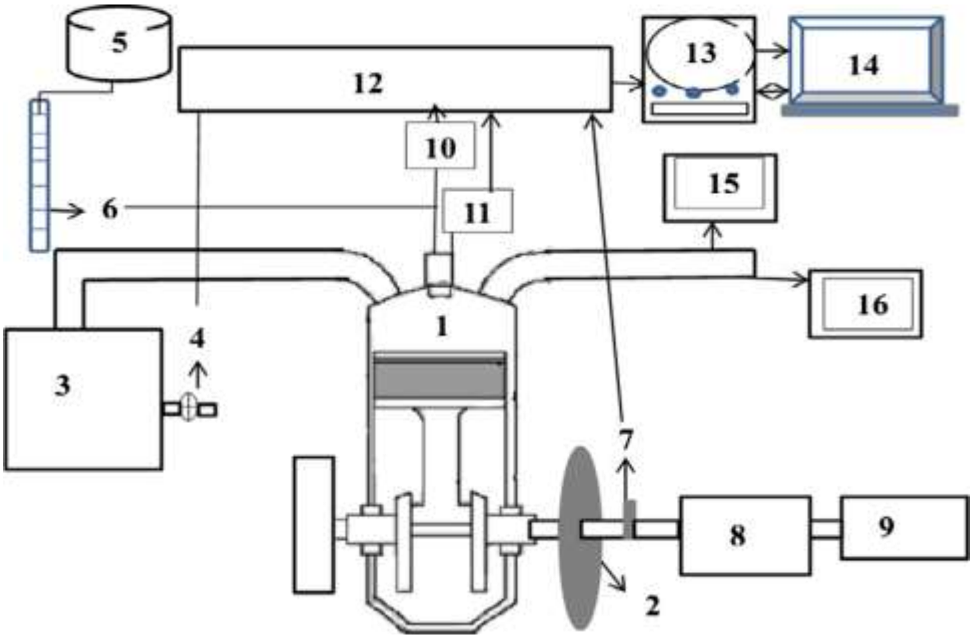
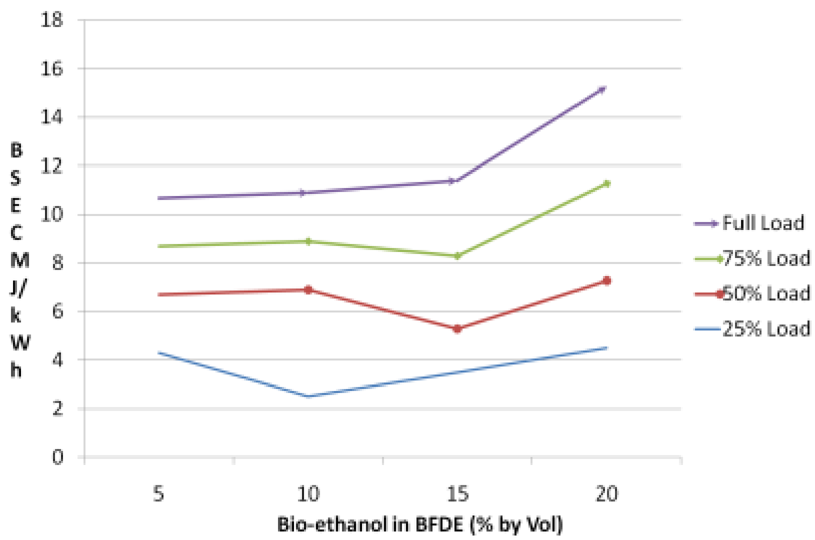


Fig-1A: Schematic diagram of Experimental setup

1. Engine; 2. Flywheel; 3. Air box; 4. Manometer; 5. Fuel tank; 6. Burette; 7. Speed sensor; 8. Alternator; 9. Loads cell; 10. Fuel injector; 11. Pressure transducer; 12. Control panel board; 13. Data acquisition card; 14. Computer; 15. AVL 437C Smoke meter; 16.AVL Digas 444 analyzer

Graph-9. Variation in BSEC for diesel and the bio-ethanol–diesel emulsions at different loads



Performance and Emission of an On IC Engine

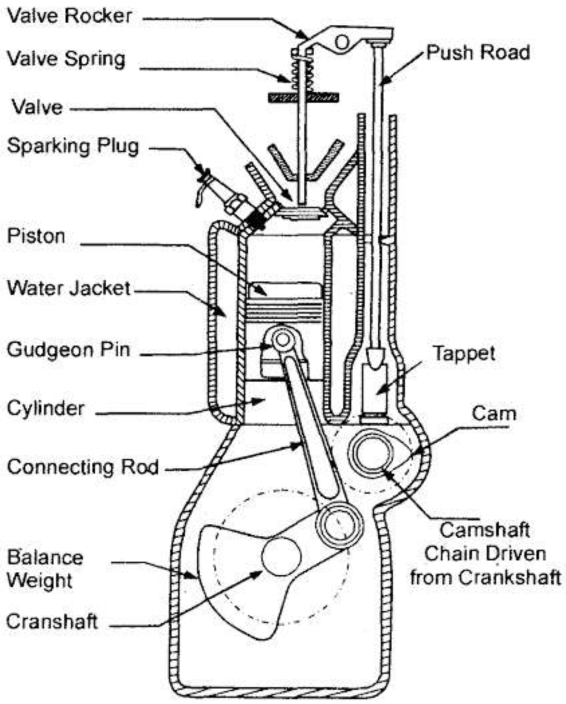


Fig.1B. Machinery components of Internal Combustion Engine

The internal Combustion Engine system with standard specification was employed to evaluate performance of the Engine with variable blends of Bio-ethanol and Petroleum Diesel (Fig.1B & 2 and Table-12). In addition, the mechanism of action with the CI engine during the supply of fuel was enunciated (Fig.2&3). The Effect of ethanol–gasoline blends on CO and HC emissions in last generation four stroke CI engines within the cold-start transient was evaluated, which works with ethanol-gasoline mixture by 5%, 10%, 15%, 20%, and 30% by volume called G5, G10, G-15, G20, and G30. This study showing that, the reduction of the CO and HC emission at cold-start transient commercial gasoline was observed, with the range of 10%, 15% to 20% v/v ethanol blend achieving the highest emission decrease (Table-13).

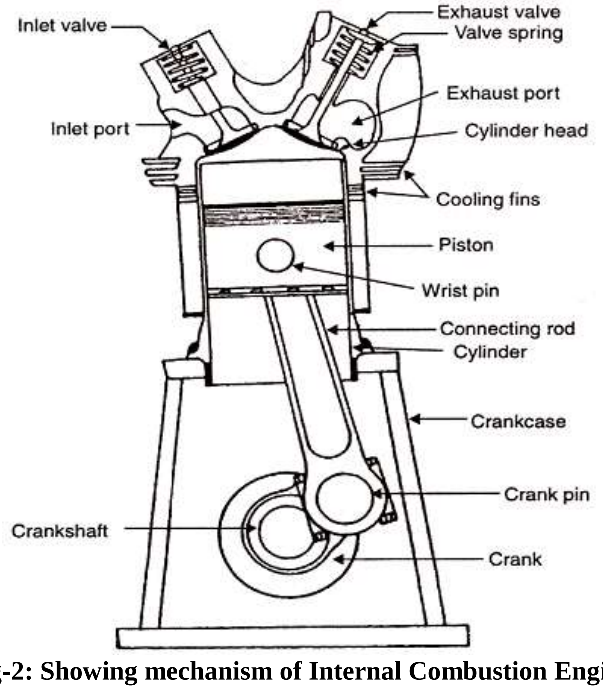


Fig-2: Showing mechanism of Internal Combustion Engine

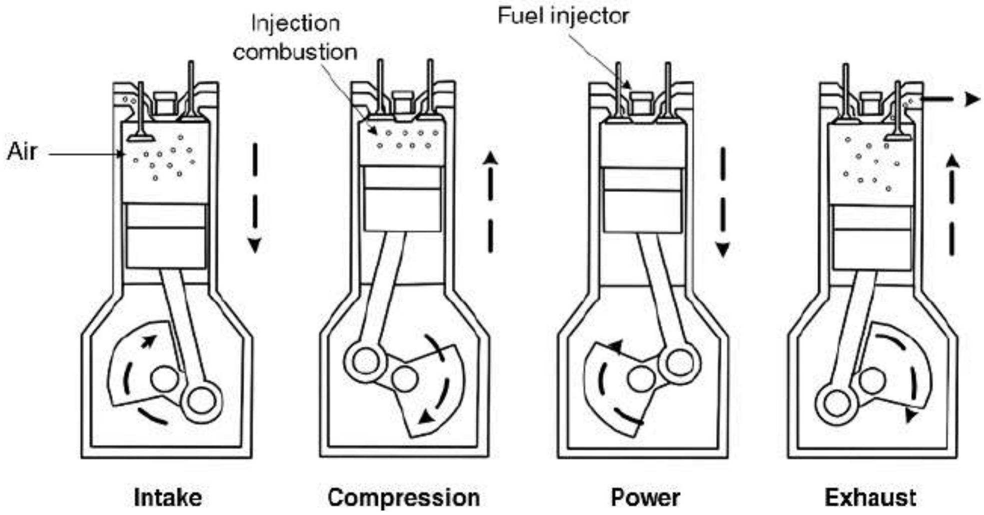


Fig-3: Showing mechanism of action Internal Combustion Engine during fuel supply

The burning of fossil fuel and emission of hazardous gases are measured as keeping this factor as a major cause of global warming and environmental pollutions [40]. The ethanol produced from fruit resources was tested by using the CI Engine of above arrangement. In the evaluation, the hydrocarbon content for fuel consumption (ml/sec) was measured at 100% gasoline, B5 (A blend of 5% bio-ethanol/95% gasoline) and B10 (A blend of 10% bio-ethanol/90% gasoline). In the Table 4, the hydrocarbon content in B5 and B10 were found to considerably significant as compared to Gasoline alone with hydrocarbon. This showed that, the fuel blend was burned more completely in BFDE5 (*BFDE-Bio-ethanol Fuel and Diesel emulsion*) and BFDE10 as compared to the 100% gasoline permitting emission with fewer unburned hydrocarbons (HC). The noticeable performance of the engine and declined emission of gases were strongly supporting the quality of the Bio-ethanol produced by the fermentation of less edible fruits samples.

ENGINE PERFORMANCE ANALYSIS

Brake Specific Energy Consumption (BSEC)

The variation in the brake specific energy consumption of Diesel and BFDE at different loads was analyzed. The fuel consumption characteristics of an engine are generally expressed in terms of the specific energy consumption in MJ/kW h. If two different fuels of different density and calorific values are blended, then the brake specific energy consumption is considered, instead of the BSFC (Brake Specific Fuel Consumptions). It can be accomplished that the BSEC decreases with an increase in the brake power. It can was observed from the Graph-9; that the BSEC for the BFDE5, BFDE10, and BFDE15 emulsions is found to be higher by about 17.26%, 17.5%, and 30.12%, respectively, than that of diesel at full load. This may be due to the lowering of the energy content of the emulsions caused by the presence of water.

Thermal Energy balance

The useful work increases as the load increased, but other losses are decreased. The useful work for the BFDE5, BFDE10, and BFDE15 emulsions was found to be higher by about 3%, 4%, and 6%, respectively. At full load, the heat loss through the exhaust is found to be higher for all the emulsions compared to that of diesel. Though there was an efficient work is achieved by the emulsions, some amount of heat was lost, which may be due to the burning of fuels at the end of expansion stroke. Besides, the other losses are minimized with higher percentage of bio-ethanol operation (Table-14).

ENGINE EMISSION ANALYSIS

Brake Specific Hydrocarbon (BSHC) emission

The variation in the BSHC emissions with respect to different percentages of bio-ethanol in BFDE at different loads was noticed. It was observed that, the BSHC emissions of the three emulsions are found to be higher than that of diesel at full load. The BSHC emissions of BFDE10 and BFDE15 were found to be higher by about 8.33% and 22.16%, compared to that of diesel at full load. This may be due to the fact that the increase in the volume of bio-ethanol increases the heat of evaporation and formation of a quench layer. This might slows down the vaporization and mixing of fuel with air, and hence, the BSHC emissions for a given power output increased with an increase in the percentage of bio-ethanol in the emulsions. The BSHC values of diesel, BFDE5, BFDE10, and BFDE15 at full load were recorded as 0.025, 0.0279, 0.0323, and 0.0368 g/kWh, respectively, at full load (Table-13 & 15).

Table-13: Details of devices used in the analysis of Bio-fuel with CI Engine

SL. No.	Device used in the study	Reference Range	precision	Improbability (%)
1.	Load Indicator (W)	250-6000	±10	0.25
2.	Temperature Indicator (^oC)	0-900	±1	0.16
3.	Speed sensor (rpm)	0-10,000	±10	1.2
4.	Burette (cc)	1-30	±0.2	1.8
Exhaust Gas analyzer				
1.	NO (ppm)	0-5000	±50	0.8
2.	HC (ppm)	0-20,000	±10	0.75
3.	CO (%Vol)	0-10	±0.03	1.2
4.	Smoke Meter (%)	0-100	±1	1.6
5.	Pressure Transducer (bar)	0-110	±1	0.25
6.	Crank angle encoder (^oCA)		±1	0.6

Table-14: Showing thermal energy balance at full load for Bio-ethanol and Diesel emulsions

SL. No.	Parameters (%)	BFDE5	BFDE10	BFDE15	DIESEL
1.	Useful Work	35.841	37.656	39.224	32.226
2.	Heat to Exhaust	18.240	183941	19.565	17.880
3.	Heat carried by Lubricating Oil	17.660	17.202	17.409	16.676
4.	Un accounted losses	29.556	29.885	30.014	33.845

Table-15: Emission Parameters in IC Engine system

SL. No.	Type of Fuel	Oxygen content (wt.%)	CO Cold extra emissions (g)	HC Cold extra emissions (g)
1.	BFDE5	5	12.50	2.25
2.	BFDE10	10	10.25	1.45
3.	BFDE15	15	8.5	1.30

Eventually, the combustion, performance and emission characteristics of bio-ethanol with diesel fuel on a single-cylinder, four strokes, direct injection and water cooled diesel engine was assessed productively. This study gives the comparative measures at significant level with respect to brake specific fuel consumption, brake power, brake thermal efficiency, mechanical efficiency, volumetric efficiency, CO, CO_2, HC, NOx and smoke opacity. The Bio-ethanol blended at 5%, 10%, 15% and 20% ratio with diesel fuel was making possible to go with bio-ethanol at slighter blending ratios for effective commercial implementation. The results also indicated that, the CO and HC emissions were lower than diesel at 15% of MSO, and NOx emissions decreased up to 20.6% for 15% MSO when compared with diesel. From the investigation; it was accomplished that; bio-ethanol can be recommended as an alternative to diesel in a compression ignition engine without any engine modifications (Table-13).

Summary and Conclusion

The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used on routine basis for the production of fuel, electricity and other goods. The excessive consumption of fossil fuels, particularly in large urban areas, has resulted in the generation of high levels of pollution during the last few decades. The level of greenhouse gasses in the earth's atmosphere has drastically increased. With the expansion of human population and increase of industrial prosperity, global energy consumption also has increased gradually.

Importing of transport fuel is being affected by limited reserves of fossil fuel. The annual global oil production will begin to decline within the near future. In this scenario, renewable sources like, bio-ethanol from sustainable bio-resources might serve as an alternative to petro-diesel.

Further, the other form of renewable energy sources like, wind, water, sun, biomass, geothermal heat can also be explored positively for the benefit of energy industry whereas; fuel production and the chemical industry may conceivably depend on biomass as an alternative source in the near future. Besides, all the petroleum-based fuels can be replaced by renewable biomass fuels such as bio-ethanol, bio-diesel, bio-hydrogen, biogas etc., derived from Cereals, millets, sugarcane, Fruit cultivars, algae, potential bio-resources etc.

In the present study, the unbolt technologies were adopted for bio-ethanol production from less edible fruit cultivars; Passion fruit and Cashew apple fruit samples. The bio-ethanol content of both Passion fruit and Cashew apple fruit juice samples by employing fermentation with increased duration using flocculating Yeast was successfully achieved. The result bring to a close outcome that, the bio-ethanol content of Passion fruit by yeast fermentation was significantly very high compared to Cashew apple fruit sample. Furthermore, other than the yeast fermentation, in the ethanol production, using flocculating Yeast culture can effectively facilitates high amount of bio-ethanol recovery in the fruit samples with 100% purity. Besides, the results also showed that, the bio-ethanol obtained in Passion fruit is of highest purity and can be recommended directly to use as fuel ethanol.

In the study, gasoline was taken as reference which is blended with bio-ethanol. The physical properties relevant to the fuel were determined for the four blends of gasoline and bio-ethanol. A standard engine test rig with varying rpm, Petrol engine connected to eddy current type dynamometer was run on blends containing BFDE- 5%, 10%, 15%, 20% ethanol respectively and the performance characteristics were evaluated. It can be concluded that using 10% bio-ethanol blend exclusively is most effective and it can be recommended for use directly in CI engines with petite restraint on material used to sustain significant increase in pressure. Further, the spark ignition engine with bio-ethanol and the performance and emission analysis was found to be noteworthy and the reduction on the emission of CO, NO_X, CO_2 was recorded. Hence blending of bio-ethanol at about 10-20 % can lead to a better performance of engine as compared with pure diesel.

ACKNOWLEDGEMENT

The authors are very much grateful to; the Bhoomigeetha Institute of Research & development (BIRD), Tumkur (Karnataka), INDIA for providing infrastructure and Dept. of Biotechnology, SIET, Tumkur (Karnataka), India. The Manager of Azyme Biotechnologies, Bengaluru for training and technical assistance with statistical analysis and the authorities of Bharathiar University, Coimbatore (Tamilnadu) for providing opportunity to pursue the proposed Research study.

REFERENCES

1. Goh, C. S., & Lee, K. T. (2010). "A visionary and conceptual macroalgae- based third-generation bio-ethanol (TGB) bio-refinery in Sabah, Malaysia as an underlay for renewable and sustainable development," Renewable and Sustainable Energy Reviews, vol. 14, no. 2, pp. 842–848.
2. Jegannathan, K. R., Chan, E. S., & Ravindra, P. (2009). "Harnessing biofuels: a global Renaissance in energy production?" Renewable and Sustainable Energy Reviews, vol. 13, no. 8, pp. 2163–2168.
3. Walker, G. M. (2011). "Fuel ethanol: current production and future challenges," Journal of the Institute of Brewing, vol. 117, no. 1, pp. 3–22.
4. Amigun, B., Sigamoney, R., & von Blottnitz, H. (2008). "Commercialization of bio-fuel industry in Africa: a review," Renewable and Sustainable Energy Reviews, vol. 12, no. 3, pp. 690–711.
5. Demirbas, A. (2008). "Bio-fuels sources, bio-fuel policy, bio-fuel economy and global biofuel projections," Energy Conversion and-Management, vol. 49, no. 8, pp. 2106–2116.
6. Julius, E., & Lena, T. (2013). "Cluster Development in Low Resource Settings: the Case of Bio-ethanol and Fruit Processing Clusters in Uganda". Journal of Entrepreneurship and Innovation Management, Vol. 2(3), 59-78.
7. Bigelow, M., & Wyman, C. E. (2002). Cellulase production on bagasse pretreated with hot water. *Applied biochemistry and biotechnology*, *98*(1-9), 921-934.
8. Kimand, S. D. BE 2004.". *Global potential bioethanol production from wasted crops and crop residues," Biomass and Bio-energy*, *26*(4), 361-375.
9. Anderson, W. F., Akin, D. E. (2008). Structural and chemical properties of grass lignocelluloses related to conversion for biofuels. *J Ind MicrobiolBiotechnol*. 35:355–366.
10. Omojasola, P. F., Jilani, O. P., & Ibiyemi, S. A. (2008). Cellulase production by some fungi cultured on pineapple waste. *Nature and Science*, *6*(2), 64-79.
11. Galbe, M., & Zacchi, G. (2012). A review of the production of ethanol from softwood. *Appl Microbiol Biotechnol* 59:618–628.
12. Janani, K., & Ketzi, M. (2013). Comparative studies of ethanol production from different fruit wastes using *Saccharomyces cerevisiae*. Journal of Innovative Research in Science, Vol. 2, 12.
13. Fatma, H. A., & Fadel, M. (2010). Production of bio-ethanol via enzymatic saccharification of rice straw by cellulase produced by *Trichoderma reesei* under solid state fermentation. *New York Sci J*. 3: 72-78.
14. Bhandari, S. V., Panchapakesan, A., Shankar, N., & Kumar, H. A. (2013). Production of bioethanol from fruit rinds by saccharification and fermentation. *International Journal of Scientific Research Engineering & Technology*, *2*(6), 362-365.
15. Balat, M., & Balat, H. (2009). Recent trends in global production and utilization of bio-ethanol fuel. *Applied energy*, *86*(11), 2273-2282.
16. Turner, D., Xu, H., Cracknell, R. F., Natarajan, V., & Chen, X. (2011). Combustion performance of bio-ethanol at various blend ratios in a gasoline direct injection engine. *Fuel*, *90*(5), 1999-2006.
17. Honorato, T. L., Rabelo, M. C., Gonçalves, L. R. B., Pinto, G. A. S., & Rodrigues, S. (2007). Fermentation of cashew apple juice to produce high added value products. *World Journal of Microbiology and Biotechnology*, *23*(10), 1409-1415.
18. Neelakandan, T., & Usharani, G. (2009). Optimization and production of bioethanol from cashew apple juice using immobilized yeast cells

by Saccharomyces cerevisiae. *American-Eurasian Journal of Scientific Research*, *4*(2), 85-88.

19. Araújo, S. M., Silva, C. F., Moreira, J. J. S., Narain, N., & Souza, R. R. (2011). Biotechnological process for obtaining new fermented products from cashew apple fruit by Saccharomyces cerevisiae strains. *Journal of industrial microbiology & biotechnology*, *38*(9), 1161-1169.
20. Pinheiro, Á. D. T., Rocha, M. V. P., Macedo, G. R., & Gonçalves, L. R. (2008). Evaluation of cashew apple juice for the production of fuel ethanol. *Applied Biochemistry and Biotechnology*, *148*(1-3), 227-234.
21. Osho, A. (2005). Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. *African Journal of Biotechnology*, *4*(7), 660-662.
22. Van Zyl, W. H., Lynd, L. R., den Haan, R., & McBride, J. E. (2007). Consolidated bioprocessing for bioethanol production using Saccharomyces cerevisiae. In *Biofuels* (pp. 205-235). Springer, Berlin, Heidelberg.
23. Panjai, L., Ongthip, K., & Chomsri, N. (2009). Complex fruit wine produced from dual culture fermentation of pineapple juice with Torulaspora delbrueckii and Saccharomyces cerevisiae. *Asian Journal of Food and Agro-Industry*, *2*(2), 135-139.
24. Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. *Applied microbiology and biotechnology*, *69*(6), 627-642.
25. Alfenore, S., Molina-Jouve, C., Guillouet, S., Uribelarrea, J. L., Goma, G., & Benbadis, L. (2002). Improving ethanol production and viability of Saccharomyces cerevisiae by a vitamin feeding strategy during fed-batch process. *Applied Microbiology and Biotechnology*, *60*(1-2), 67-72.
26. Dos Santos Lima, F. C., da Silva, F. L. H., Gomes, J. P., & da Silva Neto, J. M. (2012). Chemical composition of the cashew apple bagasse and potential use for ethanol production. *Advances in Chemical Engineering and Science*, *2*(04), 519.
27. Deenanath, E. D., Rumbold, K., & Iyuke, S. (2013). The production of bioethanol from cashew apple juice by batch fermentation using Saccharomyces cerevisiae Y2084 and Vin13. *ISRN Renewable Energy*, *2013*.
28. Jeffery, R. W., Baxter, J., McGuire, M., & Linde, J. (2006). Are fast food restaurants an environmental risk factor for obesity?. *International Journal of Behavioral Nutrition and Physical Activity*, *3*(1), 2.
29. Gritli-Linde, A. (2007). Molecular control of secondary palate development. *Developmental biology*, *301*(2), 309-326.
30. Heilemann, M., Van De Linde, S., Schüttpelz, M., Kasper, R., Seefeldt, B., Mukherjee, A., ... & Sauer, M. (2008). Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes. *Angewandte Chemie International Edition*, *47*(33), 6172-6176.
31. Rocha, M. V. P., Oliveira, A. H. S., Souza, M. C. M., & Goncalves, L. R. B. (2006). “Natural cashew apple juice as fermentation medium for bio-surfactant production by *Acinetobacter calcoaceticus*,” *World Journal of Microbiology and Biotechnology*, vol. 22, no. 12, pp. 1295–1299.
32. Pacheco, A. M., Gondim, D. R., & Gonçalves, L. R. B. (2010). Ethanol production by fermentation using immobilized cells of Saccharomyces cerevisiae in cashew apple bagasse. *Applied biochemistry and biotechnology*, *161*(1-8), 209-217.
33. Hector, R. E., Mertens, J. A., Bowman, M. J., Nichols, N. N., Cotta, M. A., & Hughes, S. R. (2011). Saccharomyces cerevisiae engineered for xylose metabolism requires gluconeogenesis and the oxidative branch of the pentose phosphate pathway for aerobic xylose assimilation. *Yeast*, *28*(9), 645-660.
34. Canilha, L., Chandel, A. K., Suzane dos Santos Milessi, T., Antunes, F. A. F., Luiz da Costa Freitas, W., das Graças Almeida Felipe, M., & da Silva, S. S. (2012). Bioconversion of sugarcane biomass into ethanol: an overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. *BioMed Research International*, *2012*.
35. Mishra, A., & Bäuerle, P. (2012). Small molecule organic semiconductors on the move: promises for future solar energy technology. *Angewandte Chemie International Edition*, *51*(9), 2020-2067.
36. El-Diwany, A. I., El-Abyad, M. S., El-Refai, A. H., Sallam, L. A., & Allam, R. F. (1992). Effect of some fermentation parameters on ethanol production from beet molasses by Saccharomyces cerevisiae Y-7. *Bioresource technology*, *42*(3), 191-195.
37. Bellissimi, E., & Ingledew, W. M. (2005). Metabolic acclimatization: preparing active dry yeast for fuel ethanol production. *Process Biochemistry*, *40*(6), 2205-2213.
38. Najafi, G., Ghobadian, B., Tavakoli, T., & Yusaf, T. (2009). “Potential of bio-ethanol production from agricultural wastes in Iran,” Renewable and Sustainable Energy Reviews, vol. 13, no. 6-7, pp. 1418–1427.
39. Balasubramanian, K., Ambikapathy, V., & Panneerselvam, A. (2011). Studies on ethanol production from spoiled fruits by batch fermentations. *Journal of Microbiology and Biotechnology Research*, *1*(4), 158-163.
40. Partha, D. (2008). Global Warming, greenhouse gases and their harmful effects – Urgent reduction of these are essential to save our environment.