

Characteristics and evolution of bioethanol from Manila Tamarind (*Pithecellobium dulce*) leaf through fermentation

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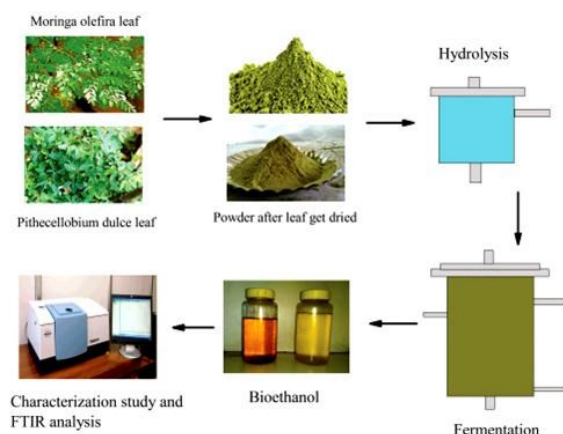
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Graphical abstract



Abstract

This study's two primary goals are to synthesize bioethanol from biowaste and examine its thermal characteristics. Bioethanol must first undergo a comprehensive assessment of its thermal characteristics in order to be approved for usage in spark-ignition engines. A multi-step procedure comprising extraction, pretreatment, enzymatic hydrolysis, and fermentation was used to manufacture the bioethanol. The raw material was put through a preliminary screening using thermogravimetric analysis to find the mass loss rate as a function of temperature before to starting this process. Remarkably, the Manila tamarind leaves exhibited the highest mass loss, up to 34%. Enzymatic cellulose conversion to fermentable sugars was a critical step in the production of cellulosic ethanol. Following hydrolysis, *Saccharomyces cerevisiae* was employed in the fermentation process, leading to the bioethanol synthesis phase. The Manila Tamarind leaves yielded the most, around 29% by weight, which is amazing. In order to assess the thermal properties of the extracted ethanol, a variety of parameters were carefully examined, including the viscosity, density, cetane number, and calorific value. In each of these areas, mixtures of tire oil, gasoline, and bioethanol consistently outperformed the others. Furthermore, Fourier Transform Infrared Spectrometry spectra were utilized to validate the concentrations of potential groups, including alcohol,

aromatic, alkyne, amide, and carbonyl groups. Clear objectives guide the fermentation process, aiming for consistent quality, safety, and functionality in the end product.

Keywords: Bio ethanol, Fermentation, FTIR, Hydrolysis, Manila Tamarind Leaf, TGA

1. Introduction

The rapid global proliferation of alternative fuels has led to a significant decline in the availability of fossil fuels. To curb the consumption of fossil fuels, numerous researchers are exploring alternative options [Chandran *et al.*, 2017]. Our study concentrated on the creation of bioethanol that could be produced from various bio sources and combined with gasoline for use in spark-ignition (SI) engines. Global bioethanol output rocketed from 13,800 million gallons in 2006 to 26,520 million gallons in 2019. The majority, around 80% of the total production, took place in the USA and Brazil, primarily using corn and sugarcane [Kang *et al.*, 2014].

Distinguishing industrial ethanol from bioethanol, the former is typically produced by hydrating ethylene with acid in a petrochemical process. In contrast, in oxygen-depleted environments, microorganisms like bacteria or yeast, such as *Zymomonas mobilis*, ferment various bio sources to yield bioethanol [Kang *et al.*, 2014]. While second-generation ethanol is made from lignocellulosic sources, first-generation ethanol is created from feedstocks having a sugar foundation. Algal feedstock is used to produce third-generation ethanol [Kang *et al.*, 2014]. Bioethanol boasts favorable fuel properties for SI engines, thanks to its high octane number, approximately 99, compared to regular gasoline's octane rating of about 91. However, its low cetane number, bioethanol cannot be used in compression ignition engines. When paired with a cetane booster like an emulsifier, it can occasionally be used in such engines [Shen *et al.*, 2011]. A similar peak at 1050 cm⁻¹ was found when the samples' FTIR spectra were analyzed, demonstrating the materials' susceptibility for igniting [Lachenmeier, 2007].

Bioethanol was produced using a variety of ingredients, including sugar cane juice, raffia wine, palm wine, and malt

beverages made from maize. Among these, sugarcane juice yielded the most ethanol, approximately 44.87 cl/kg. To evaluate the performance of each ethanol sample when blended with gasoline, a SI engine was employed, demonstrating improved performance with a wide range of ethanol-gasoline mixtures [Tangka *et al.*, 2011]. This experiment underscores the potential benefits of blending palm and *Jatropha* biodiesel with three innovative additions. When substituting diesel engine fuel with biofuel, specifically coconut oil, the key emphasis is on emission control and engine performance. The study investigates the performance of a small diesel engine using an experimental setup that incorporates different blends of biodiesel derived from coconut oil. The engine demonstrates smooth and efficient operation with biodiesel. Various biodiesel blends have been employed to avoid the need for significant modifications to the engine or fuel supply system, ensuring its seamless integration [Kanthavelkumaran *et al.*, 2011].

Regarding the investigation of spray characteristics using ethanol and gasoline combinations, it was observed that the spray pattern remained relatively consistent. However, as the concentration of ethanol in the mixture increased, there were contrasting changes in the spray tip's penetration and angle [Gao *et al.*, 2007]. A researcher concentrates on highlights how yeast may readily ferment the peels by enzymatic hydrolysis. To sum up, the information gathered about fermentation in lignocellulose wastes showed that enzymatic hydrolysis suggests that the peels may be used as an alternate feedstock and are a financially viable option for the manufacture of bioethanol [Kanthavelkumaran *et al.*, 2023].

Furthermore, an alternative fuel resembling gasoline, derived from used engine lubrication oil and waste tires, was tested in a spark-ignition (SI) engine. Both the pure gasoline-like fuel and its blends exhibited lower brake fuel consumption and improved thermal efficiency [Demirbas, 2008; Demirbas, 2009]. Tire oil, which is produced from sand (52 vol%) and TiO₂, showed the highest oil output from tire waste [Chandran *et al.*, 2017]. Similar to this, the maximum oil output from grape seeds was obtained using a combination of pressure shock wave induction and enzyme hydrolysis procedures. Individual underwater shake pretreatment and enzyme hydrolysis, as reported by Marouek *et al.* in 2015 [Maroušek *et al.*, 2015], enhanced oil extraction by 10% and 16%, respectively. These efforts are expected to contribute to the development of energy self-sustainability strategies in developing countries, particularly those based on bioethanol. By concentrating just on changes in the concentrations of biofuels, a diminished impact is expected in the context of environmental health. In both industrialized and emerging nations, like India, using biofuels as fuel for internal combustion engines may be extremely important in reducing the negative environmental effects produced by fossil fuels. Overall, researchers have shown that using biodiesel is critical to creating a more environmentally friendly climate [Kanthavelkumaran *et al.*, 2016].

The production of bioethanol relies on essential processes, including fermentation and hydrolysis. Commercially, two common hydrolysis methods involve the use of concentrated acid and diluted acid. Enzymatic hydrolysis, utilizing cellulolytic enzymes, functions similarly. Lignin removal or pretreatment is necessary to expose the hemicelluloses and cellulose molecules and alter the crystalline structure of fiber. In this study, concentric acid hydrolysis was employed to break down fiber and hemicelluloses.

2. Materials and methods

The approximate chemical make-up of Manila tamarind leaves is shown in Table 1. The dried-up leaf powder was used for the proximate analysis. The Indian state of Tamil Nadu's different district is where the leaves were gathered. No specialized equipment was needed for collection since only a small quantity of leaves was required for the batch-type reactor; thus, manual harvesting sufficed. Following collection, the leaves were sun-dried for an additional three days during daylight hours before being crushed into powder using a four-bladed crushing mill.

The multi-step procedure for bioethanol synthesis typically involves the different key stages were Feedstock Preparation, Hydrolysis, Fermentation, distillation, dehydration, blending and storage.

2.1. Feedstock Preparation:

Biomass, such as corn, sugarcane, or lignocellulosic materials, is collected and processed to extract fermentable sugars.

2.2. Hydrolysis:

Hydrolysis is a chemical process in which a molecule is split into two parts by the introduction of a water molecule. This reaction is prevalent in biological, chemical, and industrial settings. In biological systems, enzymes often catalyze hydrolysis reactions, such as the breakdown of complex carbohydrates into simpler sugars during digestion. Chemically, esters can undergo hydrolysis to produce carboxylic acids and alcohols. Industrial applications include saponification, where esters react with alkalis to form soap and glycerol, as well as the hydrolysis of starch in food processing. Hydrolysis also finds use in environmental applications for treating pollutants, and it plays a role in metallurgy, polymer degradation, and the breakdown of certain metal salts. Understanding the specific context or application allows for a more detailed exploration of hydrolysis-related processes.

2.3. Fermentation:

Microorganisms, usually yeast (e.g., *Saccharomyces cerevisiae*), ferment the sugars to produce ethanol and carbon dioxide. This step converts sugars into alcohol.

2.4. Distillation:

The fermented mixture undergoes distillation to separate and concentrate the ethanol from the fermentation broth, removing impurities.

2.5. Dehydration

Water is removed from the concentrated ethanol through processes like molecular sieves or azeotropic distillation to increase ethanol purity.

2.6. Blending and Storage:

Ethanol may be blended with gasoline in various proportions to produce ethanol-blended fuels, such as E10 or E85. The final product is then stored for distribution and use.

Table-1 Approximate composition of the bio-source.

	Crude lipid (wt %)	Crude protein (wt %)	Ash (wt %)	Moisture (wt %)	Crude fiber (wt %)
Manila Tamarind leaf	6.54	21.36	17.54	7.27	4.92
Moringa oleifera leaf	7.12	24.23	15.4	7.01	6.45

To transform the leaves into powder, they were drawn between the crushing blades through suction. The blades were positioned closer together, and they spun at a faster rate, approximately 1500 rpm, resulting in the leaves being finely pulverized. The crushing mill had a capacity of 50 kg/h and operated at 2 kW. To maintain pressure within the reactor throughout the hydrolysis process, a 1.5 kW single-cylinder reciprocating compressor was also used. Energy costs are a significant factor in the production of alternative fuels. For this research, a batch-type manufacturing unit was utilized. Approximately 18% of the total production costs were allocated to the purchase, operation, and maintenance of the compressor. Additionally, if a plant were to produce 18.6x10⁶ liters annually, this cost could potentially be reduced by up to 25%.

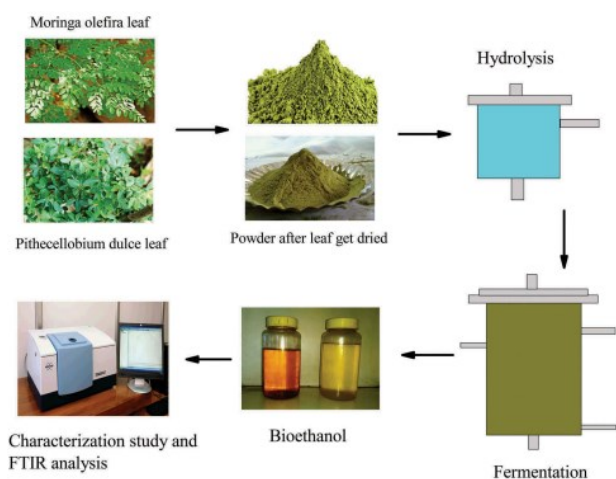


Figure 1. Process Flow Chart

The sample powders underwent thermo-gravimetric analysis (TGA) and proximate analysis to determine their energy content prior to the biological process. Figure 1 depicts the hydrolysis of hydrochloric acid-containing water, which was followed by fermentation that might last up to 10 days. To make the produced ethanol purer, it underwent a distillation procedure. Regarding the use of SI engines, the thermal characteristics investigation and FTIR study were conducted.

TGA specifications include the following:

Model: SDT Q600.

Nitrogen and air are required for operation. Specifications for FTIR include the following:

Model: Tensor 27, Bruker Optik GmbH, Germany

Spectral range 375-7550 cm⁻¹

3. Results and discussion

3.1. Thermogravimetric analysis

Thermogravimetric Analysis (TGA) is a technique used to study the weight changes of a sample as a function of temperature (or time) in a controlled atmosphere. There are several specific reasons for conducting Thermogravimetric analysis:

3.2. Determination of Thermal Stability:

TGA helps in assessing the thermal stability of materials by observing weight loss or gain as a function of temperature. It is particularly useful for identifying the temperature at which a material begins to degrade or undergo chemical reactions.

3.3. Characterization of Decomposition Processes:

TGA can provide insights into the decomposition processes of materials. By analyzing the rate and extent of weight loss, researchers can identify the stages and mechanisms of decomposition.

3.4. Evaluation of Purity:

TGA can be employed to assess the purity of a substance. Impurities or contaminants in a sample may exhibit different thermal behaviors compared to the main component, allowing for their detection and quantification.

3.5. Study of Reaction Kinetics:

TGA data can be used to study reaction kinetics by analyzing the rate of weight change over time. This information is valuable for understanding the kinetics of decomposition, oxidation, or other chemical reactions.

3.6. Quality Control in Industries:

In industries such as polymers, pharmaceuticals, and food, TGA is used for quality control purposes. It helps in monitoring the consistency and stability of materials and ensuring they meet specified standards.

3.7. Polymer Analysis:

TGA is widely used in the study of polymers. It provides information on thermal transitions, such as glass transition temperatures and melting points, as well as degradation temperatures. This information is crucial for designing and processing polymers.

3.8. Catalyst Studies:

TGA can be applied to study catalysts and catalytic reactions. It helps in understanding the catalytic activity, stability, and the effects of temperature on catalyzed reactions.

3.9. Selection of Processing Conditions:

TGA is used to optimize processing conditions for various materials, such as determining the suitable temperature range for a manufacturing process without causing degradation.

3.10. Environmental Analysis:

TGA is employed in environmental studies to investigate the thermal behavior of materials in different atmospheres. This can be relevant for applications such as waste treatment and disposal. TGA can provide valuable insights into the thermal stability, composition, and decomposition characteristics of materials under various environmental conditions. TGA in environmental analysis plays a crucial role in understanding the thermal characteristics of materials relevant to environmental science, waste management, and climate change studies. It provides valuable information for optimizing processes, ensuring material compatibility, and assessing the environmental impact of various substances.

3.11. Material Compatibility Studies:

TGA is useful for assessing the compatibility of different materials in composite systems or multicomponent formulations, providing insights into potential interactions or reactions. TGA in material compatibility studies is a valuable tool for assessing how materials respond to temperature variations and environmental conditions. This information is critical for designing and selecting materials that can maintain their stability and performance in specific applications.

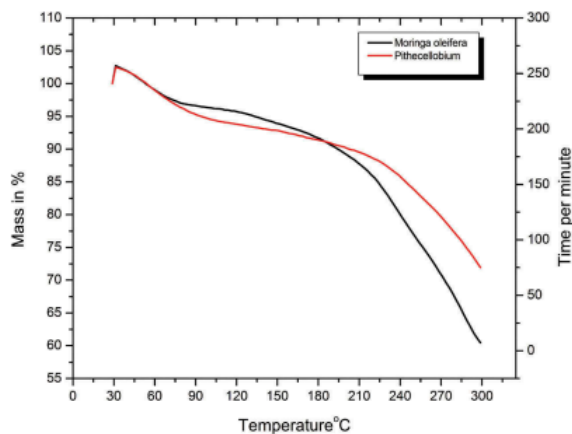


Figure 2. TGA of Bio sources

In the analysis, a five-milligram sample was subjected to heating at a rate of 30°C per minute while being analyzed under a nitrogen atmosphere. Figure 2 illustrates that mass loss commenced when the temperature was in the range of 30°C to 36°C. At an estimated temperature of 86% of the entire mass, loss was about 5%. The greatest mass loss at 310°C reached about 38% of the entire mass, indicating a considerable rate of mass loss. Both before and after being heated to 100°C, samples' moisture and other small components were effectively removed, resulting in a potential reduction of up to 5% in weight. The remaining volatile material, accounting for approximately 36% of the total weight, progressively vaporized up to 310°C.

3.12. Production Yield

Figure 3 displays the conversion rate over the course of the fermentation period. In both samples, the initial conversion rate ranged from 20 to 30 ml/kg. The highest output occurred on the fourth day of fermentation, averaging between 80 and 95 ml/kg. By the end of the tenth day of fermentation, however, the production had decreased to 30 to 40 ml/kg. Utilizing moringa oleifera leaf, the greatest renovation rate was about 36% by weight. It's important to note that the generated bioethanol's thermal characteristics and FTIR spectra were found to be identical.

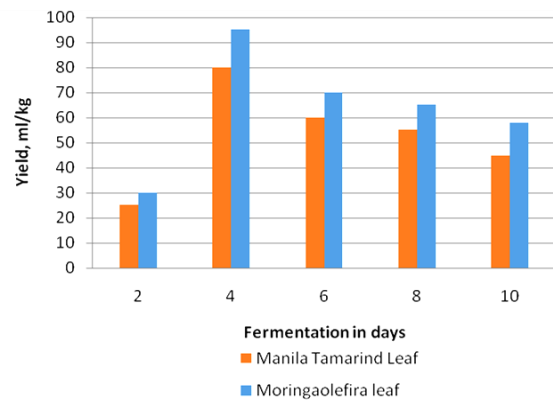


Figure 3. Ethanol Conversion Yield

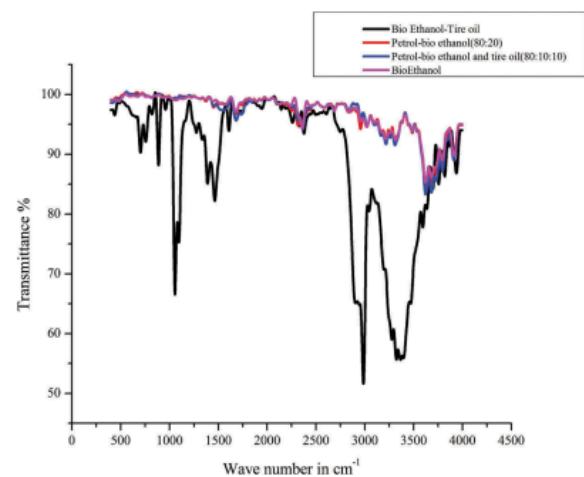


Figure 4. FTIR spectra for various ethanol samples

Table 2 provides a cost analysis for batch-type manufacturing, where transportation accounts for 18% of the total costs, while the hydrolysis and fermentation processes contribute to 32%. In the event of a bigger bioethanol plant, such as one that produces 1000 liters per month, these expenses may be decreased by up to 20%.

The hydrocarbon fuel known as tire oil is created from old tire scraps using either thermal or catalytic pyrolysis at temperatures between 360°C and 500°C. It was often employed for its higher carbon content and used in desulphurization and distillation processes to remove sulfur. During the pyrolysis process, the majority of the oil, between 55 and 60% by volume, was generated. Distillation was conducted within the temperature range of 140 to 400 degrees, and approximately 75-80% of the extractions during distillation constituted liquid fuel, while 4-5% were pyrogens, and the remainder was in the form of sludge [Chandran *et al.*, 2017]. In this study, a mixture

consisting of 50% by volume of distilled tire oil and bioethanol was used.

3.13. FTIR spectra analysis

A summary of the numerous functional groups found in the various blended samples is shown in Figure 4. In every sample, many prominent peaks were found:

Peaks between 3600 and 3900 cm^{-1} are signs that there is free alcohol present. Wide peaks between 3300 and 3700 cm^{-1} are indicative of alcohol with a (O-H) hydrogen bond. The presence of primary amine (N-H) groups is indicated by peaks between 3400 and 3700 cm^{-1} . The presence of aromatic compounds (C-H) is confirmed by a medium-intensity peak between 3000 and 3100 cm^{-1} . Alkanes (C-H) are indicated by strong peaks between 2800 and 3000 cm^{-1} .

Peaks between 1350 and 1480 cm^{-1} , suggesting the presence of alkanes with variable bonding. Strong alkenes (=C-H) between 675 and 1000 cm^{-1} . The FTIR spectra indicated that the bioethanol band was bent in each case, and it was evident that tire oil had a strong bond between 1000 and 1400 cm^{-1} , indicating the presence of (C-F) alkyl halides. Furthermore, tire oil and bioethanol mixes may include amine (C-N) based on a weak strained bond at 1116 cm^{-1} . The presence of either in mixed tire oil sample ranged from 1070 to 1150 cm^{-1} . Nitrile (CN) was found in pure bioethanol and bioethanol-gasoline mixes at values ranging from 2210 to 2250 cm^{-1} .

The samples had considerably greater amounts of aromatic content, alcohol, and alkane when tire oil was present, as shown by the FTIR spectra. Additionally, it was discovered that the samples' amounts of alcohol, alkyne, carbonyl, and amide were increased by bioethanol. FTIR spectrometry, with its ability to provide detailed molecular information, is a valuable tool for validating concentrations of potential functional groups in a wide range of applications, including chemical analysis, pharmaceuticals, polymers, and environmental monitoring. The validation of concentrations of specific functional groups in synthesized bioethanol is integral to ensuring the overall quality of the product. This process not only meets regulatory standards but also impacts the economic feasibility, safety, and environmental sustainability of bioethanol production. Validating concentrations provides a comprehensive assessment of the bioethanol suitability for various applications, promoting its successful integration into diverse industries.

3.14. Thermal properties analysis

The **density** of bioethanol can vary based on factors such as temperature, ethanol content, and any additives present in the fuel.

Pure Ethanol (at 20 degrees Celsius):

The density of pure ethanol at 20 degrees Celsius is approximately 0.789 grams per milliliter (g/mL).

E10 (10% Ethanol, 90% Gasoline):

For a common ethanol-gasoline blend known as E10 (containing 10% ethanol and 90% gasoline), the density is typically slightly higher than that of pure gasoline due to the higher density of ethanol. The density of E10 can be around 0.76 to 0.77 g/mL.

E85 (85% Ethanol, 15% Gasoline):

In the case of E85 (85% ethanol and 15% gasoline), the density is significantly lower compared to pure gasoline. The density of E85 can be in the range of 0.78 to 0.79 g/mL.

Kinematic viscosity is a measure of a fluid's resistance to flow under the influence of gravity. It is defined as the ratio of dynamic viscosity to density.

The **Gross Calorific Value (GCV)** of a substance, commonly a fuel, is a measure of its heating value or energy content when completely combusted. It represents the total amount of heat released during the combustion process, including the heat released by condensation of water vapor formed during combustion. The unit of measurement is typically expressed in energy units such as joules per unit mass or energy per volume.

Table 2. Cost study of bioethanol production in batches.

Transportation (in INR)	Hydrolysis and fermentation process (in INR)	Crushing (in INR)	Separation (in INR)	Total (in INR)
104/ton	148/ton	92/ton	112/ton	448/ton

The **octane number** is a measure of the resistance of a fuel to knocking or pinging in internal combustion engines. Knocking is an undesirable phenomenon that occurs when the fuel-air mixture in the engine's combustion chamber ignites spontaneously and unevenly before the spark plug fires. It can lead to engine damage and reduced efficiency.

There are two types of octane numbers: Research Octane Number (RON) and Motor Octane Number (MON). These are determined through standardized tests.

Research Octane Number (RON) is determined under milder conditions than MON and is representative of the fuel's performance under light to moderate driving conditions. The RON is usually higher than the MON for a given fuel.

Fuels with higher RON values have better anti-knock properties. For example, a fuel with RON 95 has better resistance to knocking than a fuel with RON 90.

Motor Octane Number (MON) is determined under more severe conditions, simulating high-speed driving conditions. It represents the fuel's resistance to knocking under harder acceleration and higher engine loads. The MON is generally lower than the RON for the same fuel.

The Anti-Knock Index (AKI) is an average of the RON and MON and is commonly used in North America. It is calculated using the formula:

$$\text{AKI} = (\text{RON} + \text{MON}) / 2$$

The octane rating of a fuel is usually displayed at fuel stations, and it is important for consumers to use a fuel

with the appropriate octane rating for their vehicle. High-performance or high-compression engines often require fuels with higher octane ratings to prevent knocking.

Basic guidelines for octane ratings:

Regular unleaded gasoline typically has an octane rating of 87.

Mid-grade or plus gasoline usually has an octane rating around 89-90.

Premium gasoline has a higher octane rating, often 91 or higher.

The **cetane number** is a critical parameter used to assess the ignition quality of diesel fuel in internal combustion engines. It serves as an indicator of how readily the fuel ignites in a diesel engine, influencing combustion characteristics and overall engine performance. A higher cetane number suggests better ignition quality, indicating that the fuel ignites more easily under the conditions found in diesel engines. This leads to shorter ignition delays, smoother combustion, reduced engine noise, enhanced fuel efficiency, and lower emissions. Standardized testing methods, such as ASTM D613, are employed to determine the cetane number by comparing the fuel's ignition delay with that of reference fuels. Diesel engines may have varying cetane number requirements for optimal performance, and the cetane index provides a quick estimation of the cetane number based on other fuel properties. Overall, the cetane number plays a crucial role in ensuring the efficient and reliable combustion of diesel fuel in internal combustion engines.

Table 3. Characteristics of several samples.

	Density kg/m ³	Kinematic viscosity CST	Gross calorific value in kJ	Motor octane Number	Research oc- tane number	Cetane number
Test methods	IS 1448 P 32	IS1448P25	Bomb colorimeter	IS 15607: 2005		
Petrol/Gasoline	776	0.7	41,229.14	89	95	9
Bioethanol	816	1.8	27,568.34	91	104	13
Petrol/gasoline Bio- ethanol + (80 + 20)	801	1.16	35,584.14	89	96	8
Bioethanol+ tire oil (70 + 30)	854	2.5	34,367.1	79	84	21
Petrol/gasoline+ bio- ethanol + tire oil (80 + 10 = 10)	818	1.5	36,897.0	86	93	16

Table 3 provides an overview of the basic thermal properties of ethanol samples and their blends: Gasoline has a higher density compared to pure ethanol and its mixtures. The higher density of bioethanol suggests that it contains more energy. Pure bioethanol has slightly higher kinematic viscosity than gasoline, and this viscosity increases further when blended with distilled tire oil. Ethanol exhibits a lower gross calorific value compared to gasoline. More calories are present in oil.

The calorific value is an essential factor that indicates a fuel's ability to burn. It's worth noting that gasoline has a higher calorific value than pure bioethanol. The octane number is a critical factor in assessing a fuel's ability to prevent detonation and knocking during combustion. Research and motor octane values, along with other measures, indicate that bioethanol has a higher octane number than pure gasoline. Similarly, samples with the highest cetane level included tire oil. While many studies have focused on using tire oil in diesel engines due to its higher cetane number compared to diesel fuel, your study

emphasizes the use of bioethanol and tire oil as alternative fuels blended for gasoline engines. These thermal properties provide insights into the energy content, combustion characteristics, and suitability of different fuel combinations for various engine types.

4. Conclusion

In our region, we've successfully achieved bioethanol production from Manila Tamarind leaves, achieving an impressive conversion rate of 31%. To enhance transportation efficiency, it is advisable to compress the leaves into briquettes before commencing the biological processing. An examination of the FTIR spectra demonstrates that the presence of bioethanol enhances the occurrence of functional groups like alcohol, alkyne, carbonyl, and amide in the samples. Additionally, when analyzing samples of distilled tire oil, we observe indications of aromatic rings, alcohol, and alkane groups, akin to what is observed in blended samples. Furthermore the incorporation of bioethanol into gasoline has been demonstrated to be compatible with spark-ignition (SI) engines, allowing for operation at levels of up to 20 vol% without necessitating any modifications. However, challenges may arise when dealing with blends with viscosities surpassing 1.6 CST at levels exceeding 20 vol%, affecting the pumping and mixing processes. In such cases, making minor adjustments to the fuel pump and carburetor becomes essential to ensure the sustained use of bioethanol blends in SI engine operations over extended periods. This parallels the suitability of introducing tire oil into bioethanol blends at a rate of approximately 10% for SI engine operations.

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