




UTILIZATION OF BEEF TALLOW IN THE SYNTHESIS OF ETHYL ESTER NITRATE TO IMPROVE DIESEL OILJoice Manga¹ * , Ridhawati¹ , Jeanne Damayanti¹ ¹Department of Chemical Engineering, Politeknik Negeri Ujung Pandang, Makassar, IndonesiaCorresponding author email: joicemanga@poliupg.ac.id**Article Info**

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Abstract

This study aims to test the use of beef fat as a raw material in the manufacture of ethyl ester nitrate, which can be used as an additive to increase the cetane number in diesel oil. In this case, beef fat is converted into biodiesel ethyl ester first through a transesterification reaction process with a mole ratio of beef fat and ethanol of 1: 13 and then converted again into ethyl ester nitrate through a nitration reaction using a homogeneous catalyst of Sulfuric Acid and Nitric Acid reagent. The mole ratio in the nitration reaction between ethyl ester and nitric acid is 1: 1. The ethyl ester nitrate product obtained is then mixed into diesel oil in several concentrations, namely 0.5%; 1% 1.5% and 2%. Then, performance testing is carried out on a diesel engine. The results of the analysis with FTIR show that ethyl ester nitrate has been successfully synthesized from biodiesel ethyl ester. Through performance testing on diesel engines with parameters of engine power (Q_{tot} , kWatt), braking power (BHP, kWatt), fuel consumption (FC, kg/h), specific fuel consumption (SFC, kg/kWh), mean effective pressure (MEP, kPa), and thermal efficiency (η_{th}) and exhaust emission measurements. Interpretation of data from this experimental analysis, it has been proven that ethyl ester nitrate 1.5% v/v can play a good role as a bio additive for diesel oil because it can increase the efficiency of diesel engines to produce better exhaust emissions and save fuel (SFC) around 34% with a higher braking power of 40% at 2000 RPM engine speed, although it slightly reduces engine power. Although the engine becomes more wasteful of fuel, the provision of ethyl ester nitrate compounds with a concentration of 2% v/v in diesel oil also needs to be considered because it can provide very high engine power and braking power.

Keywords: Beef tallow, Cetane Improver, Diesel Oil, Ethyl Ester Nitrate

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INTRODUCTION

The use of diesel as a diesel engine fuel causes environmental problems due to flue gas emissions. To reduce the rate of air pollution it causes, improvements need to be made to the diesel engine itself and or its fuel. One way that can be done to reduce flue gas emissions is to use fuels that have a high cetane number. Fuel cetane figures are known to correlate directly with the ignition quality of the diesel engine;

the higher the value, the shorter the time delay for ignition so that the amount of fuel needed is a little bit. In general, high cetane numbers can increase engine efficiency, reduce flue gas emissions, and the amount of heat lost (Ramalingam et al., 2018).

Increasing the cetane number of diesel fuel can be done, for example, by mixing diesel fuel with certain compounds that have high cetane numbers, either in small amounts (for example, 2-EHN additives, essential oils) (R. Sathiyamoorthi et al., 2018, Lu et al., 2024, Kuszewski & Jaworski, 2024) or in large amounts (for example, biodiesel) (Velmurugan & Sathiyagnanam, 2016, Agumsah et al., 2023). In other references, additives can be synthesized from mixing nanoparticles in fuel. Generally indicate increasing BTE from 1 to 25%, except for magnesium and carbon nanotube additives, which can reduce BTE by up to 4.8%. Hydrocarbon emissions generally decrease from 4 to 60%, while NO_x emissions generally decrease from 4 to 45%, except for manganese, aluminum oxide (Ali Ijaz Malik et al., 2024, Bitire et al., 2023)

In practice, the use of small amounts of additives mixed into diesel oil is the most practical choice. Unfortunately, existing additives are generally products of the non-renewable petrochemical industry. To reduce dependence on these non-renewable energy sources, intensifying the search for raw material sources for alternative fuels needs to be done. Animal fats generally contain components that are dominated by triglycerides. In beef tallow, the triglycerides have a high concentration of saturated fatty acids (C16: 0 and C18: 0) and polyunsaturated fatty acids. Most animal fats consist of 50% of the total saturated fatty acids. These animal fats are stearic (Lee & Wilson, 2015) melting point and viscosity so that they are solid at room temperature. (Adewale et al., 2015, Cernat et al., 2020). As with triglycerides from vegetable oils, beef tallow triglycerides can also be further processed into oleochemical products such as cosmetics, soap, lubricants and biodiesel fuels. Besides, the difference between animal fat and some vegetable oils such as soybean oil is known from the composition of unsaturated fatty acids more than animal fat, especially beef tallow (Silvana et al., 2008, Lebedevas et al., 2006). In contrast to palm oil and other edible oils which cause debate about food for humans or engine fuel, the manufacture of bio additives from biodiesel from beef tallow can be realized without causing significant debate because beef tallow is a type of high cholesterol food and rarely consumed directly by humans (Huang & Chang, 2010). Meanwhile, alkyl esters derived from palmitic and stearic acids have cetane numbers greater than 80, while alkyl esters from oleic, linoleic and linolenic fatty acids have cetane numbers of 55 - 58, 40 and 25 respectively (Lee & Wilson, 2015).

Alkyl esters from animal fats are known to have high Cetane numbers, which is an important parameter for diesel fuel (Adewale et al., 2015). The cetane number of biodiesel from animal fat (beef tallow) feedstock is high, ranging from more than 60 because the material contains a high ratio of saturated fatty acids (Cernat et al., 2020, Toldrá-Reig et al., 2020). Higher Cetane numbers are known to reduce NO_x by lowering the temperature during the critical initial part of the combustion process. Similarly, saturated fatty acids in beef tallow contribute to better oxidative stability for biodiesel (Jonas et al., 2020). Animal fats are considered to have prospects as a raw material in biodiesel and bioadditive synthesis at a low cost (Knothe, 2005).

The availability of beef tallow, although limited, is abundant in certain regions; therefore, beef tallow has the potential as a raw material for biodiesel and additives (Toldrá-Reig et al., 2020), (García-Morales et al., 2022, Esther Olubunmi et al., 2022). In previous studies, beef tallow alkyl esters were known to have higher cetane number and lower gas emissions than soy alkyl esters, so that they were interestingly developed as additives for diesel oil (Barrios et al., 2014, Silvana et al., 2008)

RESEARCH METHOD

The research materials included ethanol (E. Merck), beef tallow, NaOH (E. Merck), H₂SO₄ (E. Merck), HNO₃ (E. Merck), and anhydrous CaCl₂ (E. Merck) and aquades. This research begins with the synthesis of esters from beef fat through a transesterification reaction pathway with ethanol reagents, followed by the synthesis of ethyl ester nitrate bioadditives from ethyl ester biodiesel through a nitration reaction pathway. Performance tests on diesel engines were carried out using the Small Engine Test Bed TQ TD115 and IC Engine Instrumentation TQ TD 114. This test is useful to determine the extent to which performance and exhaust emissions can be improved by adding ethyl ester nitrate products to diesel fuel. In addition to testing performance on diesel engines and exhaust emissions, characterization of the identification of functional groups of ethyl ester and ethyl ester nitrate compounds was also carried out

using the FTIR Spirit-X Series Shimadzu and determination of the quantity of calorific value of the Oxygen Bomb Calorimeter Brand B-One,

The data required to calculate the parameters tested on a diesel engine are as follows:

W : Fuel consumption time

Ho : Air Flow manometer

Tdb : Dry air temperature

Twb: Wet air temperature

Tgb : Exhaust gas temperature

CV : Caloric value

The performance of adding ethyl ester nitrate additives to fossil diesel oil for diesel engine performance can be determined using formulas 1 to 6.. (Veza et al., 2023, Abdillah et al., 2025).

Horsepower (HP): This measures the engine's power output. It indicates how much work the engine can perform over time. Brake horsepower (BHP) is often used to represent the engine's output before losses from auxiliary components like the alternator and water pump

$$BHP (kWatt) = \frac{2 * \pi * N * Tgb}{60000}$$

Specific Fuel Consumption (SFC): This measures the fuel efficiency of an engine design, typically expressed in pounds of fuel consumed per hour per horsepower. Lower SFC values indicate more efficient engines

$$FC \left(\frac{Kg}{h} \right) = \frac{8 * T * 3.6}{W}$$

$$SFC \left(\frac{Kg}{kWh} \right) = \frac{FC}{BHP}$$

Mean Effective Pressure (MEP): This is an average pressure in the combustion chamber during the power stroke. It is used to compare the performance of different engines and is calculated from the engine's power output and displacement

$$MEP (kPa) = \frac{60000 * 2 * BHP}{N * 0.1713}$$

Thermal Efficiency: This parameter indicates how well an engine converts the energy in fuel into useful work. It is calculated as the ratio of work output to heat input. Higher thermal efficiency means better fuel economy and performance

$$Q (kWatt) = \frac{FC * CV}{3600}$$

$$\eta_{th} (\%) = \frac{BHP}{Q} * 100\%$$

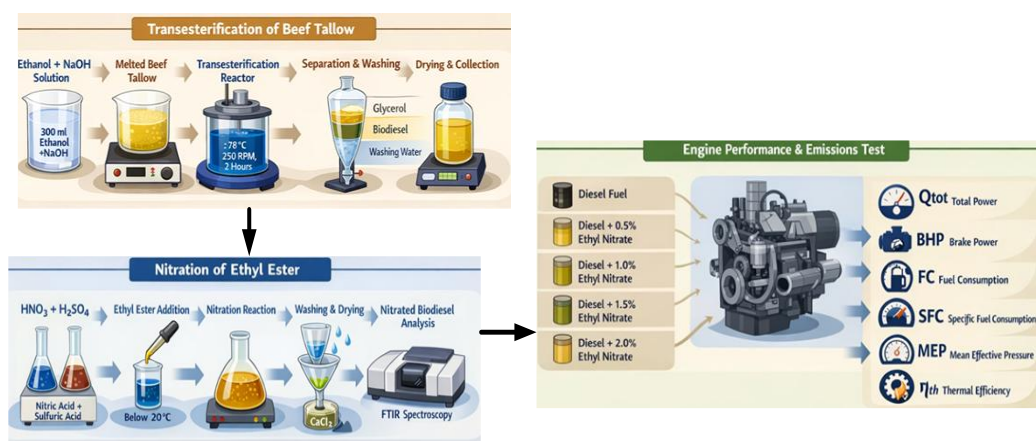


Figure 1. Schematic of ethyl ester nitrate synthesis process and application testing

Research Procedure

Transesterification Reaction Of Beef tallow. Initially 13 mol of ethanol in the reactor was activated with 0.75% w/w NaOH of the fat weight. In the beaker glass, 1 mol of fat were melted on a hot plate to a temperature of at least 80 °C (Jambulingam et al., 2020, Ghanbari Zadeh Fard et al., 2019, Verma et al., 2016, Gandure et al., 2017). After all reactants are ready, the reaction was carried out for 2 hours in the reactor at a reaction temperature of 78 °C and a stirring rate of 250 rpm (Manga & Yulistiono, 2021). After the reaction time is reached, the reactor is turned off. The liquid is removed and collected into a separating funnel. The purification process by washing was then carried out using distilled water until the pH of the wash water used was in the range of 6-7. The obtained product was then heated at 105 °C for 30 minutes to remove emulsified water.

Reaction Of Ethyl Ester This nitration reaction of biodiesel ethyl ester was carried out using nitric acid and concentrated sulfuric acid catalyst at temperatures below 20 °C and the molar ratio between ethyl ester, nitric acid and concentrated sulfuric acid catalyst is equal to 1: 1: 1. At first, the nitric acid was reacted with concentrated sulfuric acid, after that it was reacted again drop by drop with biodiesel ethyl ester (Ben Talouba et al., 2024, Canoira et al., 2007, Burov et al., 2023, Liu, 2015, Nasikin et al., 2002). After all the acid has been dropped into biodiesel, the reaction was continued for the next 1 hour. After the reaction time is complete, the reaction liquid is washed with cold water several times. To separate the remaining water in the nitration product, anhydrous CaCl₂ is used. (Nurliana et al., 2024)

Performance Test on Diesel Engines and Flue Gas Emissions. As an initial step in determining the effect of ethyl ester nitrate compounds on diesel fuels, it is necessary to analyze the diesel calorific value and its mixtures with ethyl ester nitrate at several concentrations. Performance testing and emission measurements were carried out simultaneously on 5 types of fuels, namely diesel oil obtained from public refueling station, diesel oil mixed with ethyl ester nitrate compounds as much as 0.5% v/v, 1.0% v/v, 1.5% v/v, and 2.0% v/v. The parameters tested were total engine working power (Qtot, kWatt), braking power (BHP, kWatt), fuel consumption (FC, kg/hour), specific fuel consumption (SFC, kg/kWh), mean effective pressure (MEP, kPa), and thermal efficiency (ηth) (Srinivasan et al., 2019)

RESULTS AND DISCUSSION

Fourier Transform-Infra Red (FTIR) Analysis

Identify the existence of nitrate functional groups, characterization was carried out with infra red. Interpretation of Fourier Transform Infra Red data reports as follows:

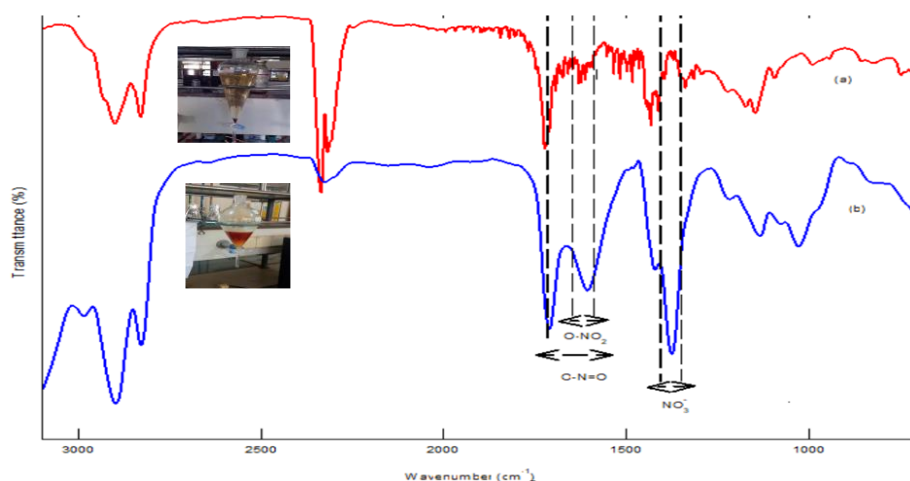


Figure 2. FTIR Spectra Of Ethyl Ester Nitrate And Ethyl Ester

The absorption band of the C-O group that identifies the ester and usually appears at the wave number 1050-1300 cm^{-1} , can be seen in Figure 2 which shows the peaks of 1018.45 cm^{-1} and 1116.82 cm^{-1} . The absorption band of the C=O group, which also identifies the presence of esters, is present at wave number 1742.74 cm^{-1} (1690-1780 cm^{-1}). For aliphatic C-H or C functional groups (-CH₂-, CH₂-CH₃), they are shown at wavenumbers 2925.15 cm^{-1} and 2853.78 cm^{-1} and usually appear at wavenumbers 2960 - 2850 cm^{-1} (Manga et al., 2020). The absorption band for nitrate functional groups is indicated at wavenumbers 859.32 cm^{-1} and 1632.8 cm^{-1} (Nurliana et al., 2024); (Diop et al., 2019), (Abdullah et al., 2017); (Gan et al., 2020); (Wu et al., 2022). Identification based on the histogram from the Fourier Transform Infra Red analysis shows that the constituent group of the ethyl ester nitrate compound has been formed.

The Calorific Value (CV, KJ/Kg)

The following is a measurement of the calorific value of some fuels made from diesel oil and ethyl ester nitrate (EEN):

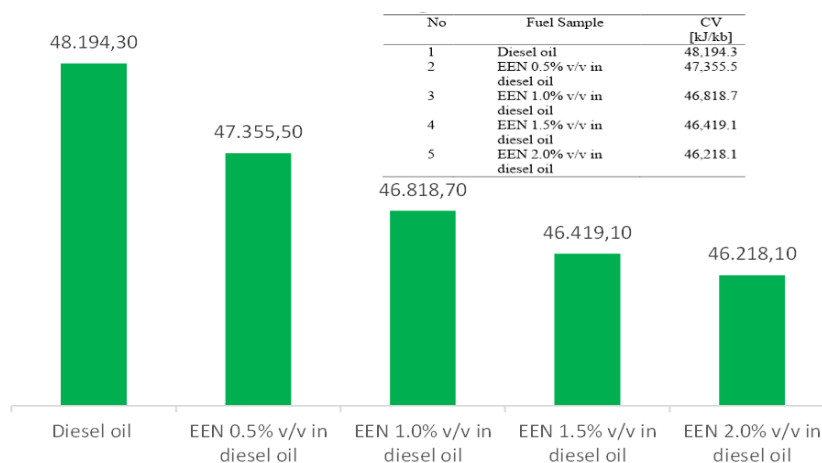


Figure 3. The Fuels Calorific Value

Figure 3 above shows that the addition of ethyl ester nitrate compounds to diesel fuel obtained from public gas stations has reduced the calorific value, where the decrease is greater with increasing amounts of ethyl ester nitrate in the diesel fuel (Permanasari et al., 2021). This is shown by the highest decrease in calorific value of around 4% at an addition of 2% (v/v).

Performance Tests on Diesel Engine

Performance tests on diesel engines have been carried out on 5 types of fuel, namely diesel oil obtained from public refueling station, diesel oil mixed with ethyl ester nitrate compounds as much as 0.5% v/v, 1.0% v/v, 1.5% v/v, and 2.0% v/v and with machine rotation at 2800, 3400, 3700, 4000 rpm.

Diesel engine performance testing was conducted on a laboratory scale, and the following data parameters were obtained::

Table 1. Testing of diesel engines using 100% Biosolar fuel

No	Throttle (%)	N (rpm)	W (detik)	ho (mmH ₂ O)	Tdb (°C)	Twb (°C)	Tgb (°C)	T (Nm)
1	25	2800	62	5,0	31	29	320	3
2	50	3400	47	5,5	31	29	360	3,5
3	75	3700	43	5,8	31	28	400	3,8
4	100	4000	38	6,0	31	28	440	4

Table 2. Testing of diesel engines using EEN 0,5% v/v + Biosolar 99,5% v/v

No	Throttle (%)	N (rpm)	W (detik)	ho (mmH ₂ O)	Tdb (°C)	Twb (°C)	Tgb (°C)	T (Nm)
1	25	2800	65	5	31	29	350	3,2
2	50	3400	54	6,5	31	29	390	3,5
3	75	3700	49	6	31	28	400	3,8
4	100	4000	40	6,5	31	28	420	4,4

Table 3. Testing of diesel engines using EEN 1,0% v/v + Biosolar 99% v/v

No	Throttle (%)	N (rpm)	W (detik)	ho (mmH ₂ O)	Tdb (°C)	Twb (°C)	Tgb (°C)	T (Nm)
1	25	2800	68	5,5	31	29	370	3,3
2	50	3400	56	6,0	31	29	410	3,5
3	75	3700	51	6,5	31	28	440	4,6
4	100	4000	45	7,0	31	28	490	4,9

Table 4. Testing of diesel engines using EEN 1,5% v/v + Biosolar 98,5% v/v

No	Throttle (%)	N (rpm)	W (detik)	ho (mmH ₂ O)	Tdb (°C)	Twb (°C)	Tgb (°C)	T (Nm)
1	25	2800	70	5,5	31	29	320	3,5
2	50	3400	59	6,2	31	29	360	4,2
3	75	3700	53	7	31	28	400	4,5
4	100	4000	48	7,5	31	28	440	5,2

Table 5. Testing of diesel engines using EEN 2,0% v/v + Biosolar 98% v/v

No	Throttle (%)	N (rpm)	W (detik)	ho (mmH ₂ O)	Tdb (°C)	Twb (°C)	Tgb (°C)	T (Nm)
1	25	2800	61	5,8	31	29	420	3,1
2	50	3400	40	6	31	29	460	4,2
3	75	3700	32	6,5	31	28	495	5
4	100	4000	28	6,8	31	28	510	5,6

This data was used to obtain diesel engine performance test parameters using ethyl ester nitrate as a bioadditive using formulas 1 to 6.

Total Working Power (*Qtot*, kWatt).

In general, in the operation of diesel engines with higher rpm *Qtot* values increase, meaning to obtain a higher engine working power, the engine rpm must be increased

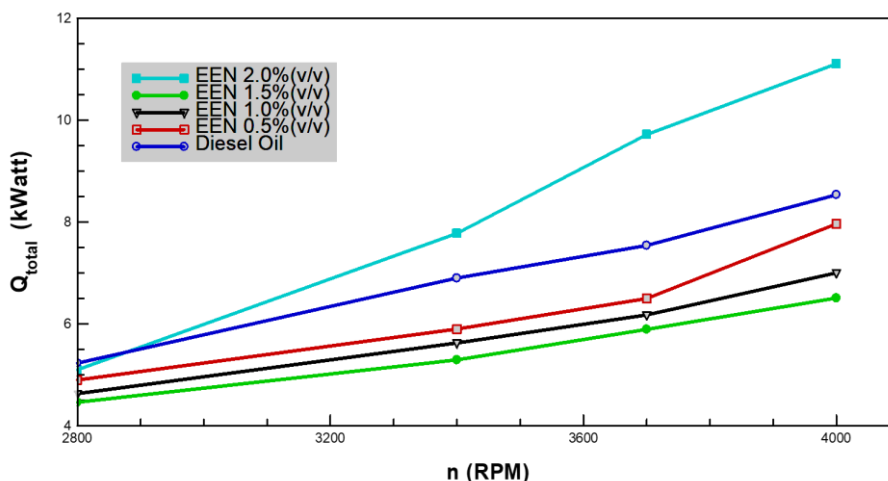


Figure 4. Relationship between Machine Work Power and Machine Rotation.

In low speed engine operations, it was seen that all fuels produce almost equal engine power (Q_{tot}), however the engine working power produced by using diesel fuel remains higher. Furthermore, it can be observed the existence of anomalies, where the addition of ethyl ether nitrate as much as 0.5% v/v, 1.0% v/v, and 1.5% v/v have led to a decrease in engine power greater, but at the addition of 2.0% v/v there was a sudden jump in the increase in engine power which was very high. This phenomenon was very strange and can not be explained, even though it has been known through experiments that have been done before, that the more the content of ethyl ester nitrate in the diesel oil, the overall calorific value of the fuel also becomes increasingly down. If the calorific value of the fuel decreases, then the engine working power must also decrease as in the addition of 0.5% - 1.5% v/v ethyl ester nitrate above. The Q_{tot} parameter test results were in the negative category except at a concentration of 2% v/v. This can occur because at this concentration according to the physicochemical characteristics of the ethyl ester nitrate compound was exothermic. In this condition, the temperature increases and Q_{tot} becomes positive.

Braking Power (BHP, kWatt)

In general, in the operation of diesel engines with a higher amount of rpm, the BHP value of a diesel engine that uses all the fuel goes up. It gives the meaning that in the operation of diesel engines with a higher amount of rpm, the diesel engine produces braking power, which is also higher

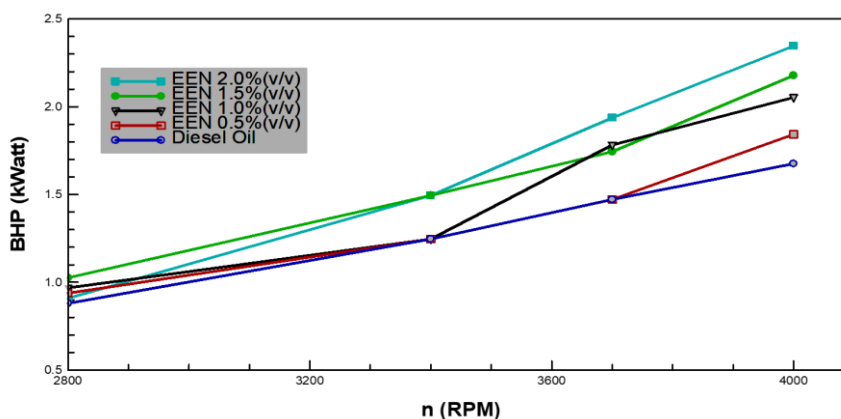


Figure 5. Relationship between BHP and Engine Speed

In low speed diesel engine operations, it was seen that the use of all fuels makes the engine have an almost equivalent BHP value, but at higher diesel engine speeds, the situation changes, where diesel oil mixed with ethyl ester nitrate compounds show their superiority. All fuels with the addition of ethyl ester nitrate, especially those at 1.5% and 2.0%, appear to be superior to diesel oil at high engine rpm. Diesel engines produced 40% greater braking power at high engine speeds when the fuel was supplemented with ethyl ester nitrate additives. The BHP parameter test results are in the positive category

(Woldetensy et al., 2025, Jayabal et al., 2023).

Consumption of Fuel per Hour (FC, Kg/Hr)

In general, in an operational engine with a higher rpm, the FC value of the fuel will rise, meaning that more fuel is needed to reach a higher engine speed.

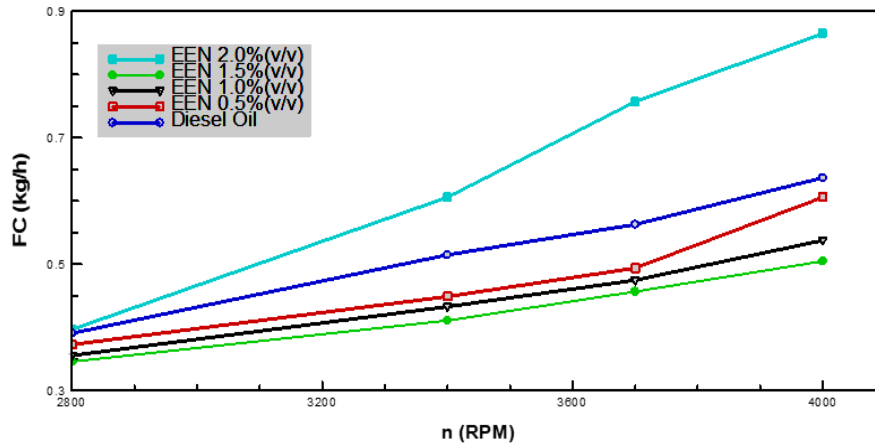


Figure 6. Relationship between FC and Engine Speed

At all engine speeds, the more the content of additive ethyl ester nitrate in the fuel up to 1.5% v/v, the lower the FC value. Even so, with the use of a 2.0% v/v additive, the FC value increased dramatically. Based on an evaluation of Q_{tot} (engine power), BHP (braking power), and FC (fuel consumption per unit time), it can be concluded that the addition of a maximum of 1.5% v/v ethyl ester nitrate can reduce fuel consumption per unit time, has more braking power good but the working power of the engine is slightly reduced. With ethyl ester nitrate as much as 2% v/v, the engine's working power and braking power become very large, but the engine becomes wasteful of fuel.

Specific Fuel Consumption per kWatt (SFC)

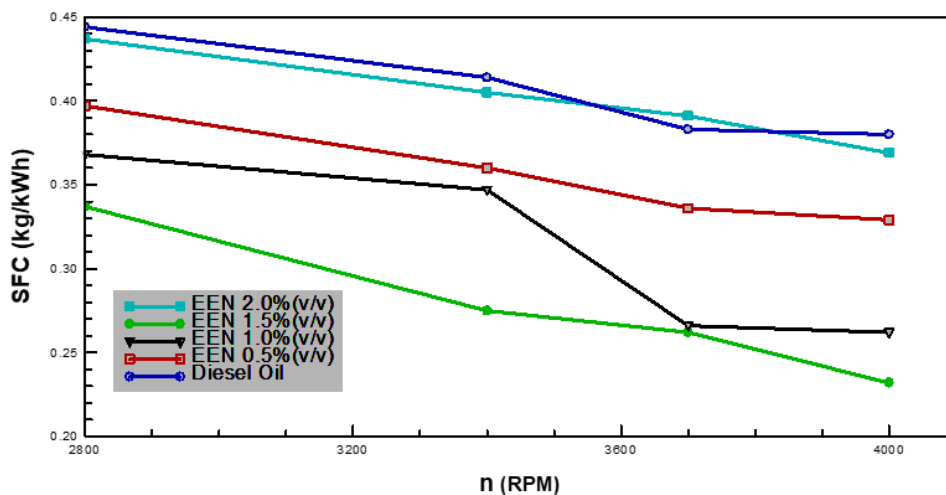


Figure 7. Relationship between SFC and Engine Speed

In general, in the operation of diesel engines with higher rpm levels, the SFC value decreases, meaning that in operational conditions with a higher engine speed, to produce performance of 1 kWatt requires less fuel, in other words faster rotation the engine the less fuel needs. The SFC value of the engine that uses only diesel fuel looks higher compared to the SFC value of the engine that uses diesel fuel which is given the additive of ethyl ester nitrate. This means, that diesel engines with diesel fuels need more fuel to obtain the same performance. So the addition of ethyl ester nitrate to diesel fuels in this case has been proven to be able to save fuel needs in diesel engines at all levels of engine speed. The maximum amount of additive that achieves optimal fuel savings is around 34% compared to diesel. Specifically, the blending ratio is 1.5% v/v ethyl ester nitrate. Furthermore, above this limit, while providing greater engine power

and braking power, fuel consumption becomes significantly wasteful.

Mean Effective Pressure (MEP)

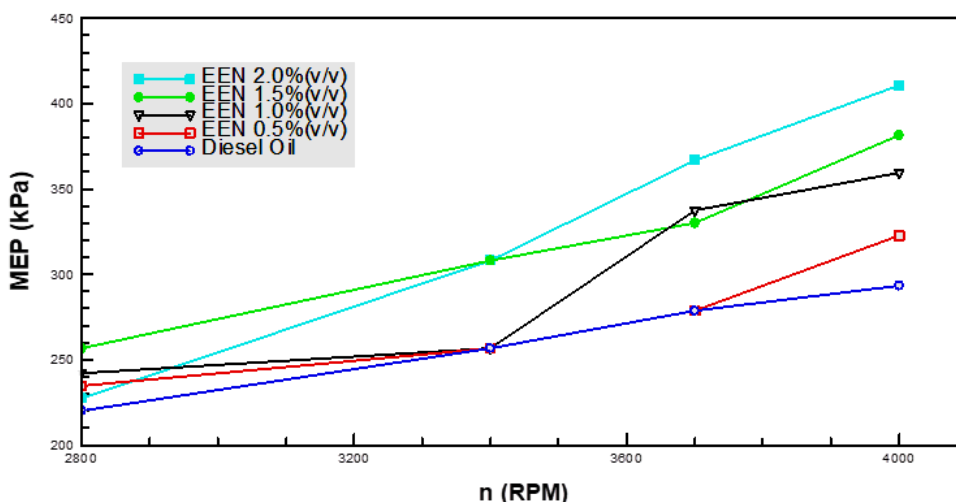


Figure 8. Relationship between MEP and Engine Speed

In general, in the operation of diesel engines with higher rpm levels, the MEP value of fuel increases, meaning that to obtain a higher effective engine pressure, the engine rpm must be increased. In low speed engine operations (2800 rpm), diesel oil has the lowest MEP value compared to other fuels, and at high engine speeds, there is a significant increase in pressure on fuels containing ethyl ester nitrate. This is indicated by a 32% increase in pressure when mixed at 2% (v/v) with an engine speed of 3700 rpm. This increase in pressure will lead to an increase in diesel engine power.

Thermal Efficiency η_{th}

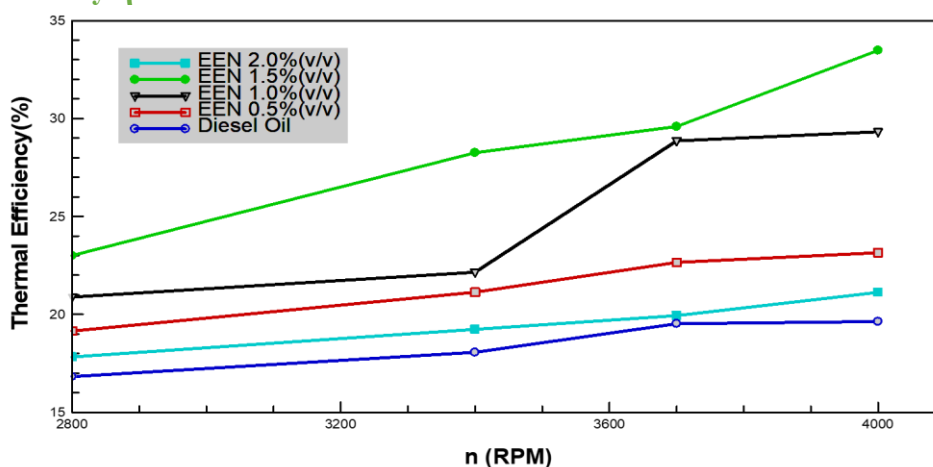


Figure 9. Relationship between Thermal Efficiency and Machine Rotation

In general, in diesel engine operations with higher rpm levels, thermal efficiency will increase, meaning that thermal efficiency will automatically increase if the engine rpm is increased (Pushparaj & Ramabalan, 2013, Xue et al., 2011). Figure 9 above shows that at all engine rotation speeds, there is an increase in efficiency if the diesel oil is given the addition of the ethyl ester nitrate compound. The optimal addition of ethyl ester nitrate occurs again at 1.5% v/v. This results in a 70% increase in thermal efficiency at the same diesel engine speed.

Measurement of Flue Gas Emissions

The following are the results of the flue gas emissions measurement tests set at 100% throttle and at 4000 rpm for diesel fuels without and with the addition of ethyl ester nitrate compound in several concentrations.

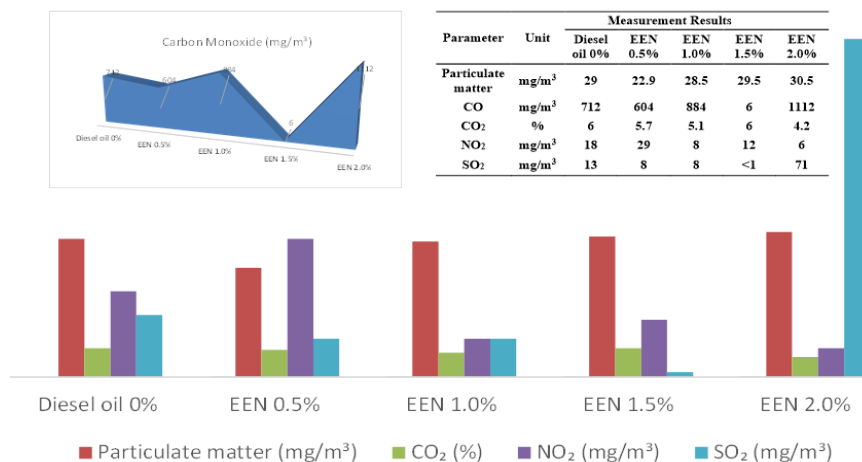


Figure 10. Flue Gas Emissions Measurement

Figure 10 above shows the effect of ethyl ester nitrate compounds on diesel oil especially about the quality of total particulate flue gas emissions, CO, CO₂, NO₂, and SO₂. The following is a brief discussion of the results of the measurement of flue gas emissions and their relationship to the amount of ethyl ester nitrate additive mixed.

Total Particulates

The addition of 0.5% v/v ethyl ester nitrate can significantly reduce the number of particulates produced by diesel engine, but at a greater concentration, the decrease in the number of particulates becomes less significant. Previous research has shown that the application of animal fat-based biodiesel results in 40% lower particulate matter emissions than from the same proportion of soybeans (Khan et al., 2024)

Carbon Monoxide (CO) and Carbon Dioxide (CO₂).

The addition of ethyl ester nitrate at 0.5%, 1%, and 1.5% v/v concentrations significantly reduced the formation of toxic CO emissions. Other research indicates that the reduction in carbon monoxide emissions in diesel engine performance increases with the addition of biodiesel additives (Xue et al., 2011), (Lv et al., 2024). Conversely, in this study, at higher concentrations (2% v/v), CO formation increased. In the results of performance tests on diesel engine, it has been mentioned that the diesel engine becomes very powerful at this concentration but is very wasteful of fuel. Maybe the combustion process becomes imperfect with the presence of too much fuel in the engine combustion chamber with also more ethyl ester nitrate content. CO₂ gas measurement data shows confusion because there is no significant effect where the formation of CO₂ gas at all concentrations of additives is almost equal. The more CO gas is formed, the less CO₂ gas formation, which indicates incomplete combustion.

Nitrogen Oxide (NO_x) As NO₂.

The use of ethyl ester nitrate at concentrations ranging from 1.0% v/v has been shown to reduce NO₂ levels in flue gases.

Sulfur dioxide (SO₂).

The sulfur content of the flue gas can be reduced reliably at the ethyl ester nitrate concentration of 1.5% v/v in the diesel oil.

CONCLUSION

Biodiesel type of ethyl ester converted from beef tallow through the transesterification chemical reaction process, can be synthesized into ethyl ester nitrate through the nitration chemical reaction process with the help of concentrated sulfuric acid catalyst. Performance tests on diesel engines have proven that the addition of a maximum of 1.5% v/v ethyl ester nitrate into the diesel oil makes the diesel engine operate more efficiently and save fuel but loses in terms of a slightly lower engine operating power. Although wasteful of fuel, ethyl ester nitrate as much as 2% v/v can provide a surge in work power and

high braking power. The flue gas emission measurement test also proves, that the addition of ethyl ester nitrate into diesel oil at a concentration of 1.5% v/v can improve the quality of diesel engine flue gas emissions.

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AUTHOR CONTRIBUTIONS

Author 1 designed and developed the research study, performed the experimental work, analyzed the data, prepared the original draft of the manuscript, and revised the article as initiator/creator and correspondent. Author 2 contributed to the development of the research methodology, supervised the laboratory analysis, and validated the research data. Author 3 assisted in the preparation of the manuscript and the interpretation of the results. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used. All aspects of the research, including laboratory work, data collection, data interpretation, and manuscript preparation, were performed entirely by the researchers, authors, and authors without the assistance of AI-based technology.

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