UNVEILING THE ANTIBACTERIAL POTENTIAL OF BIODIESEL DERIVED FROM WASTE COOKING OIL

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Abstract

Biodiesel can be made from waste materials such as used cooking oil. Each year, approximately 540,000 tons of waste cooking oil (WCO) are discarded in Malaysia without being treated as waste. However, WCO has been identified as one of the most suitable and well-known sources of biodiesel. Therefore, the goal of this research was to use WCO to produce biodiesel via the transesterification method. Due to its low cost and mild reaction conditions, transesterification is a common method. The functional group in produced biodiesel and WCO was identified using FTIR, and the antibacterial properties of yielded biodiesel were evaluated using the Kirby-Bauer test or zone of inhibition test. According to the Kirby-Bauer method, the bacteria will be inoculated onto a solid growth medium, and antibiotic discs will be introduced to the plate. This technique is used to see if pathogenic bacteria become sensitive or resistant to antimicrobial compounds found in the biodiesel sample. The FTIR results confirmed the existence of the functional group of fatty acid methyl ester (FAME) compounds in the produced biodiesel. With an inhibition zone of 8 mm, the biodiesel produced exhibited antibacterial properties. This demonstrated that the biodiesel components produced have antibacterial properties. These findings show that biodiesel derived from used cooking oil has the potential to be investigated further as antibacterial agents and to improve its performance when replacing the fossil fuels.

Keyword: antibacterial, biodiesel, fatty acid methyl ester (FAME), transesterification, waste cooking oil

Introduction

Fossil fuels have been the backbone of modern civilization, powering everything from our daily cooking routines to the massive machinery that drives industries. Yet, their usage contributes significantly to global warming by releasing greenhouse gases (Shapovalova, 2023; Wang & Azam, 2024). This realization has spurred researchers to seek alternative fuels

that can effectively replace these environmentally taxing options. Consequently, researchers are looking for new fuel alternatives that can replace fossil fuels such as bioethanol and biodiesel. Biodiesel, in particular, has gained attention for its renewable, biodegradable, and eco-friendly characteristics, mirroring the combustion traits of fossil diesel (Mahmudul et al., 2017).

Biodiesel is formed from the alcohol esters of fatty acid mixes, referred to as FAME, by a chemical reaction involving triglycerides and alcohol (Brahma et al., 2022; Suzihaque et al., 2022). This fascinating concoction opens a wide array of raw material sources, ranging from various oil feedstocks such as sunflower, soybean, and palm to unconventional sources like animal fat, microalgal lipid, and waste cooking oil (WCO) (Brahma et al., 2022). This is due to the presence of alkyl esters of fatty acids from these sources that make biodiesel a promising avenue for energy production.

Waste cooking oil has emerged as a valuable resource for biodiesel production. Studies have highlighted its viability as a source of biofuel, showcasing the environmental benefits of repurposing these waste materials into a substance that positively impacts our surroundings. Researchers have been keen on exploring innovative techniques, like using modified aluminium chloride hexahydrate with PVA as a heterogeneous catalyst or employing reactive distillation columns, to convert used cooking oil into high-quality biodiesel that adheres to industry standards (Grosmann et al., 2022; Ismail et al., 2021; Suzihaque et al., 2022).

The process of producing biodiesel from waste cooking oil predominantly involves transesterification. This method, akin to alcoholysis, replaces one alcohol with another in an ester, effectively reducing the viscosity of triglycerides (Shohaimi & Marodzi, 2018). However, the high free fatty acid (FFA) concentration in WCO poses challenges, necessitating esterification or pre-treatment to lower these levels. Acid-catalyzed and alkali-catalyzed transesterification processes have been explored, with the latter being preferred in industrial settings due to its faster reaction rates and lower corrosiveness (Suzihaque et al., 2022).

Additionally, although the antibacterial properties of biodiesel may not be the principal reason for its application, they present a supplementary advantage, especially in enhancing fuel storage stability, mitigating corrosion, and offering environmental advantages. Studies indicate that biodiesel produced from waste cooking oil may preserve certain antibacterial properties of its original feedstock, particularly if the oil contains chemicals such as phenols or other bioactive substances that impede bacterial proliferation (Dodos et al., 2017). Biodiesel with intrinsic antibacterial properties may impede the proliferation of microbes, hence potentially diminishing microbially induced corrosion in storage tanks and pipes, and prolonging the durability of infrastructure. Moreover, the combustion of biodiesel generally results in reduced emissions of hazardous substances, including sulfur oxides and particulate matter. If biodiesel maintains antibacterial properties post-combustion, it may further diminish the danger of microbial contamination in the environment, potentially decreasing the airborne transmission of specific germs. This enhances the utility of WCO in biodiesel production, functioning as both a sustainable fuel source and a medium with potential health advantages. Therefore, the aim of this study is to delve into this aspect, evaluating the purified biodiesel's effectiveness in curbing bacterial proliferation, particularly against Gram-positive bacteria, *Staphylococcus* and Gram-negative bacteria, *Escherichia coli* (*E. coli*). Techniques like the Kirby-Bauer disk-diffusion method have been employed to measure the antibacterial prowess of this purified biodiesel.

Materials and Methods

Determination of acid value by titration method

A 1 mL of phenolphthalein indicator solution and 50 mL of freshly neutralized hot ethyl alcohol were mixed together. The mixture was heated in a water bath for around 15 minutes (75–80 °C). Then, a 100 mL Erlenmeyer flask was filled with 0.1–0.3 g of fat sample. A 1-2 drop of indicator and 10 mL (*A*) of n-Hexane were added. The solution was titrated using 0.02 N potassium hydroxide (KOH) solution until light pink was observed. A blank test was performed by substituting *A* mL of C-M mixture for the extract. The acid value (AV) was calculated using the following formula Equation (1):

$$\mathrm{AV} = \frac{\mathrm{V \times N \times 56.11}}{\mathrm{W}} \quad (1)$$

where AV represents the acid value of the sample, V denotes the volume of isopropanol KOH added in mL to achieve the titration endpoint, N signifies the normality of the KOH standard solution, 56.11 represents the molecular weight of KOH, and W indicates the weight of the WCO sample in grams (Ibrahim et al., 2023).

Determination of Saponification Value

The burette was positioned on a retort stand and 0.5 M of HCl (Hydrochloric Acid) was added. The burette's stopcock was opened to titrate 2g of Al (Aluminium) into a conical flask previously filled with 25M iPKOH (Isopropyl Potassium Hydroxide Alcohol). After thoroughly mixing the sample, 5-7 drops of Polypropylene (PP) were added. The experiment was done in duplicate. The blank reading was obtained using the same method, but without the aluminum in the conical flask. The Equation (2) below is used to determine the saponification value (SV) of oil (Zulkurnain et al., 2022).

$$SV = 56.11 \times M \times \frac{(\mathrm{Vo} - \mathrm{Vi})}{\mathrm{m}} \quad (2)$$

where Vo = volume of HCl solution used for the blank test
Vi = volume of HCl solution for determination
M = actual molarity of HCl used
M = mass of sample

Transesterification method

The methanol, sodium hydroxide (NaOH) catalyst, and waste cooking oil were mixed in a reactor, stirred at 60°C for about an hour. The process occurs in two stages: first, the catalyst is added to the oil with 80 % alcohol. After removing glycerin, the remaining 20 % alcohol was then introduced into the reactor output stream. Glycerol and methyl esters separate afterward. Distilled water was added to separate glycerol, and then the methyl esters were purified through distillation or evaporation and washed with hot water. Hydrochloric acid is used to neutralize the biodiesel, taking out any leftover catalyst and soap. Though some fatty acids remain, salts are removed. To remove the remaining catalyst, salt, soap, methanol, and free glycerol from the biodiesel, washing water was used. Neutralization before washing helps avoid emulsion and reduces water needs. Lastly, any leftover water was removed by vacuum distillation.

Fourier Transform Infrared Spectroscopy (FTIR)

Using an FTIR spectrometer (Spectrum 100), the chemical changes and functional groups of waste cooking oil and biodiesel were identified. Omnic software was applied to process the collected data. The FTIR spectra were plotted against the percentage of transmittance compared to wavelength (cm^{-1}) in the 4000-400 cm^{-1} range at a resolution of 4 cm^{-1}.

Nutrient broth preparation

A 100 mL of distilled water and 0.8 g of nutritional broth powder were weighed and mixed before sealing the conical flasks with aluminium foil. The nutrient broth solutions were then autoclaved for one hour. Then, for 15 minutes, the nutrient broth solutions were cooled. The stock solution of *E. coli* and *Staphylococcus* bacteria were then introduced into the nutrient broth solution and agitated for 24 hours to let the bacteria grow.

Preparation of agar and inoculum of bacteria

A 2 g of agar powder was weighed and mixed with 100 mL of distilled water. The solution was then heated with stirring until dissolved. The solution was then autoclaved at 121°C for 15 minutes. Thereafter, six agar plates were filled with the agar solution and allowed to solidify. Then, three agar plates were each filled with 100 µL of nutrient broth containing *E. coli* using the spreading method. Three additional agar plates were then each filled with 100 µL of nutrient broth containing *Staphylococcus* using the same procedure.

Antibacterial properties of biodiesel

The sample (biodiesel), positive control (+) and negative control (-) were labeled on the agar plate. The AA disc was dipped in 30 µL of biodiesel and placed on the agar plate containing *E. coli* bacteria using sterile forceps. It was then lowered smoothly to ensure that it made contact with the agar surface. The same step was repeated on agar plates containing *Staphylococcus* bacteria. For the control test, the AA disc was dipped into the antibiotic Ampicillin and was placed in the "+" quadrant for both the *Staphylococcus* and *E. coli* plates. Meanwhile, distilled water was used as a negative control and were placed in the quadrant marked "-." The experiment was done in triplicate. All of the agar plates were incubated at 35 °C for 24 hours.

Measurement of Zone of Inhibition

After 24 hours of incubation, the presence of the zone of inhibition was observed. To ascertain the antibacterial properties of biodiesel made from WCO, the diameter of the zone of inhibition was examined and the average of the diameter was calculated.

Results and Discussion

Acid Value (AV), Saponification Value (SV) and Molecular Weight (MW) of Waste Cooking Oil

The acid value (AV), saponification value (SV) and molecular weight (MW) of waste cooking oil are shown in **Table 1**. The acid value specifies the presence of free fatty acids (FFA) in oil samples. The acid value of waste cooking oil was measured to be 6.72 mg/g.

This indicates that waste cooking oil requires additional treatment to meet the American Society for Testing and Materials (ASTM) standards in producing high-quality fuels. The saponification value is used to calculate the amount of KOH required for 1 g of fat as well as the number of esters and fatty acids in waste cooking oil. The calculated SV for waste cooking oil is 83.96 mg/g. The higher the saponification value, the longer the fatty acid chain. Waste cooking oil has a molecular weight of 2004.53 g/mol.

Table 1 The value of AV, SV and MV of waste cooking oil

AV (mg/g)	SV (mg/g)	MW (g/mol)
6.72	83.96	2004.53

Fourier Transform Infrared (FTIR)

Components in biodiesel were identified using Fourier Transform Infrared (FTIR) as shown in **Figure 1**. Carbonyl group (C=O) with a strong stretch can be found in the spectrum at 1742.37 cm^{-1}. As shown in **Figure 1**, the peak for C=O at WCO in biodiesel oil was reduced. This is similar to the research of Zulkurnain et al. (2022). Simultaneously, a peak that appeared at 1159.52 cm^{-1} was attributed to C-O stretching from the ester. The frequency range of esters (C-O) is 1361.63 to 1170.00 cm^{-1}, with a strong stretch of absorption band. Meanwhile, C-H stretching vibration for sp^3 (aliphatic) in WCO and biodiesel was assigned a peak at 2923.25 cm^{-1}. The other functional groups identified in WCO included glycerol, and alkane, with peaks at 1377.23 cm^{-1} and 721.60 cm^{-1}, respectively.

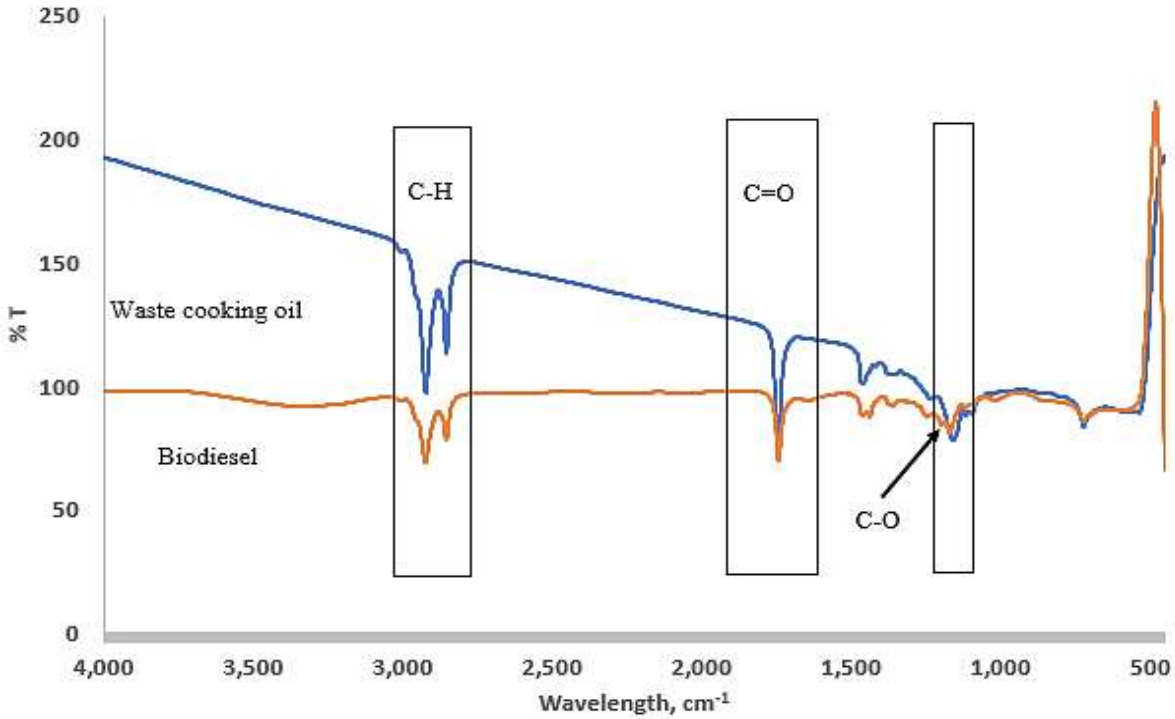

Figure 1 FTIR spectra for waste cooking oil and produced biodiesel

Antibacterial Properties of Produced Biodiesel

An antibacterial test was performed to assess the ability of the produced biodiesel to act as an antibacterial agent. **Figure 2** demonstrates that the biodiesel produced was effective against both *E. coli* and *Staphylococcus* bacteria. As shown in **Table 2**, the diameter of the inhibition zone for *E. coli* and *Staphylococcus* was 8 mm. This demonstrates that the biodiesel produced is intermediately effective between gram-negative and gram-positive bacteria. This diameter, however, was less than that of the positive control, ampicillin. This could be due to the presence of Butylated Hydroxytoluene (BHT), a phenolic-type antioxidant in WCO. According to Dodos et al. (2017) and Hossain et al. (2021), BHT, a monohydric phenol, is the least active in enhancing the antibacterial stability of FAMEs and the least efficient as an antibacterial agent when compared to other phenolic antioxidants. This is due to the fact that monohydric phenols are more soluble in lipid media than bi- or tri-hydric phenols and may tend to localize in fatty acids/material, minimizing the availability for antibacterial activity.

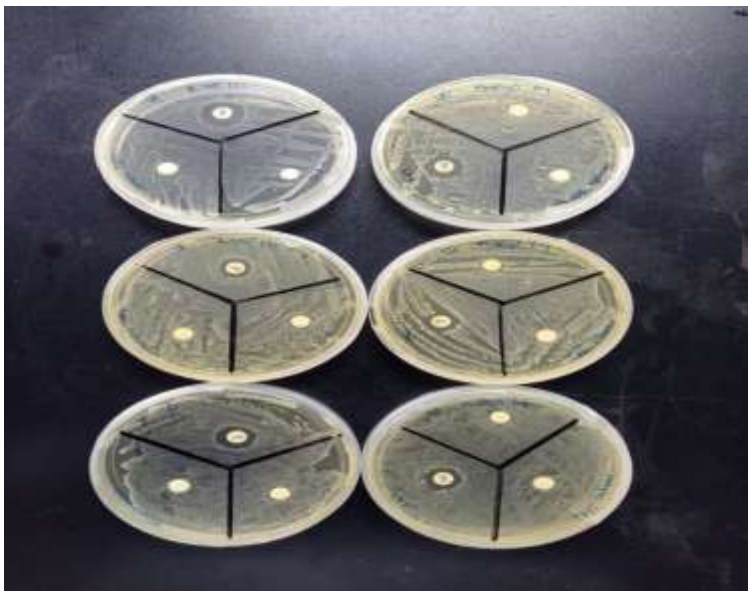

Figure 2 Zone of inhibition for biodiesel and control

Table 2 Zone of Inhibition of produced biodiesel and control

	***E. coli* (mm)**	***Staphylococcus* (mm)**
Negative control (water)	8.0	8.0
Positive control (ampicillin)	13.0	12.7
Produced biodiesel	8.0	8.0

Conclusion

To summarize, waste cooking oil was successfully converted into biodiesel. The acid value (AV) and saponification value (SV) of waste cooking oil were 6.72 mg/g and 83.96 mg/g, and the molecular weight was 2004.53 g/mol, respectively. The saponification value is calculated as the number of milligrams of KOH required to neutralize the fatty acids produced by complete hydrolysis of 1g of oil. The functional groups of the produced biodiesels were ester, carbonyl, and hydrocarbon, which were detected at 1159.52 cm^{-1}, 1742.37 cm^{-1}, and 2923.25

cm^{-1}, respectively. The functional group of waste cooking oil, on the other hand, was also identified, with glycerol and alkane, detected at peaks 1377.23 cm^{-1}, 721.60 cm^{-1}, respectively. The antibacterial properties of the formed biodiesel were determined, and the inhibition zone was observed to be 8 mm in diameter for both *E. coli* and *Staphylococcus* bacteria. Future research should focus on the treatment of waste cooking oil and the application of heterogeneous catalysts to enhance biodiesel yield and improve antibacterial properties.

Ethics Statement

The research does not require research ethics approval.

Authors Contribution

Supervision and writing-original draft preparation, Sarina Mat Rosid; Analysis data and writing-review and editing, Salmiah Jamal Mat Rosid and Norshahidatul Akmar Mohd Shohaimi; Methodology, Siti Fadhilah Ibrahim.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that might have seemingly influenced the work presented in this study.

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