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Biodiesel Production from Waste Cooking Oil via Interesterification Reaction: Effects of Cajuput Oil Biocatalyst Concentration and Reaction Time

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ABSTRACT

Interesterification processes using homogeneous or heterogeneous catalysts generally require additional separation steps, reducing efficiency and increasing operational costs. Cajuput oil, whose main active compound is 1,8-cineole, contains aromatic functional groups that show potential as a biocatalyst and can be applied without a separation step at the end of the reaction, thereby improving process efficiency. This study aims to determine the optimum conditions for the interesterification of waste cooking oil based on cajuput oil biocatalyst concentration and reaction time. Waste cooking oil, methyl acetate, and cajuput oil biocatalyst at concentrations of 0.5–1.5% were reacted in a two-neck round-bottom flask reactor at 60°C, a stirring speed of 300 rpm, an oil-to-methyl acetate molar ratio of 1:6, and reaction times ranging from 20 to 100 min. After the reaction, unreacted methyl acetate was removed by distillation, and the residue was characterized as the final product. The optimum conditions were achieved at a biocatalyst concentration of 1% and a reaction time of 60 min, producing a crude yield of 78.80%, an acid value of 0.45 mg KOH/g, a density of 0.88 g/mL, and a viscosity of 2.94 cSt, which comply with SNI 7182:2015, along with a FAME concentration of 19,406.50 mg/L. The novelty of this research lies in the biodiesel production process that eliminates the separation of the by-product triacetin and the cajuput oil biocatalyst, as both function as bio-additives in the biodiesel. Consequently, the process becomes simpler and more efficient, without requiring lengthy or complex processing steps.

INTRODUCTION

Biodiesel is an alternative fuel produced from vegetable oils or animal fats and can be used either in pure form or blended with fossil diesel fuel (Venriza & Pratama, 2023). As a renewable energy source, biodiesel consists of fatty acid ester compounds derived from various vegetable oils (Mardawati et al., 2019). Palm oil, coconut oil, jatropha oil, and kapok seed oil are examples of potential feedstocks, and more than 30 other plant species also have the potential to be developed as biodiesel sources (Manojkumar et al., 2020). Waste cooking oil (WCO) is a non-edible feedstock that is increasingly utilized as an alternative for biodiesel production to reduce competition with the food sector, which may affect food prices and availability (Prasetyo, 2018). Waste cooking oil can be processed through various methods, including transesterification, interesterification, hydrolysis, hydrodeoxygenation, hydrocracking, and hydrogenation (Youssef et al., 2024).

Biodiesel is commonly produced via a transesterification reaction between triglycerides and methanol. Although this method is effective in producing fatty acid methyl esters (FAME), it generates glycerol as a by-product. The presence of glycerol is considered disadvantageous because it requires additional handling and may reduce engine performance if mixed with biodiesel (Casas et al., 2011). To overcome these limitations, an alternative biodiesel production process has been developed by replacing alcohol reactants with non-alcohol compounds through interesterification, in which methyl acetate acts as the alkyl group donor, substituting the role of methanol in the conventional process (Casas et al., 2011). The main difference between these two methods lies in the by-products formed: interesterification produces triacetin, a value-added compound that can be used as a fuel additive or directly formulated with biodiesel, unlike glycerol, which is generally regarded as waste in transesterification processes (Casas et al., 2010; Ferreira et al., 2014).

Interesterification reactions have been widely studied using low–free fatty acid feedstocks through homogeneous base catalysts, enzymes, or non-catalytic supercritical conditions. Homogeneous catalysts are difficult to separate from the product because they dissolve in the reactants, while the preparation of heterogeneous catalysts requires lengthy pre-treatment steps prior to use (Chuepeng & Komintarachat, 2018; Daryono et al., 2021, 2022). Enzymes are reusable but relatively expensive and require specific reaction conditions. Meanwhile, supercritical methods do not require catalysts but demand high energy input and involve extreme temperatures and pressures that may degrade biodiesel quality (dos Santos Ribeiro et al., 2017; Simões et al., 2020). Recent studies have begun to explore the use of organic materials as catalysts in biodiesel production. Interesterification of waste cooking oil using 2.5% curcumin biocatalyst achieved a crude yield of 91.74% at a reaction time of 105 min (Daryono, Widi, et al., 2025).

Essential oils have emerged as alternative catalysts that are considered more efficient because they do not require a separation step at the end of the reaction. Interesterification of palm oil using eugenol as a catalyst resulted in a crude yield of 83.16% (Daryono et al., 2024) and a triglyceride conversion of 39.48% at a reaction time of 15 min (Daryono, Wardana, et al., 2025). Essential oils also function as antioxidants that enhance biodiesel stability and exhibit more environmentally friendly characteristics (Daryono & Dewi, 2022). In the interesterification of palm oil using 0.75% cajuput oil biocatalyst, the best result was obtained at the longest reaction time of 75 min, with a crude yield of 65.88% and an acid value of 0.426 mg KOH/g, meeting the requirements of SNI 7182:2015 (Daryono & Dewi, 2022). Cajuput oil has a dual role as both a catalyst that accelerates the reaction and an antioxidant that suppresses free fatty acid formation. GC–MS (Gas Chromatography–Mass Spectrometry) analysis shows that eucalyptus oil contains 71.83% 1,8-cineole, which plays an important role in providing antioxidant and antibacterial activity against both Gram-positive and Gram-negative bacteria (Quoc, 2021). The addition of eucalyptus oil to crude palm oil has also been reported to reduce viscosity, flash point, and ignition delay (Marlina et al., 2020).

Previous interesterification studies using cajuput oil biocatalyst have employed palm oil as the feedstock, which is an edible oil. In those studies, the biocatalyst concentration was treated as a fixed variable, and the optimum reaction time had not yet been achieved. The experiments were conducted using 250 g of palm oil, an oil-to-methyl acetate molar ratio of 1:6, a temperature of 60°C, a stirring speed of 300 rpm, and a catalyst mass of 0.75% relative to oil mass, with reaction times ranging from 15 to 75 min. The results indicated that the optimum condition had not yet been reached, as the yield continued to increase with longer reaction times. In that study, a commercial cajuput oil biocatalyst containing numerous impurities was used, resulting in a low content of the aromatic compound 1,8-cineole, which in turn led to a low biodiesel yield. In addition, the feedstock used was palm oil, which competes with the food industry and is therefore not recommended. Therefore, this study aims to investigate the effects of cajuput oil biocatalyst concentration and reaction time on the quality of biodiesel produced in accordance with SNI 7182:2015 (Permana et al., 2020). In this study, the biocatalyst used was cajuput oil obtained from the distillation of cajuput leaves originating from the Maluku Islands, resulting in higher purity. This research is expected to obtain more optimal reaction conditions, resulting in high-quality biodiesel that meets standards and has the potential for broader application.

RESEARCH METHODS

Equipment and Materials

The equipment used included beakers, burettes, Petri dishes, a reflux condenser, a condenser, Erlenmeyer flasks, graduated cylinders, a hot plate magnetic stirrer, a three-neck round-bottom flask, an oven, volumetric pipettes, measuring pipettes, a pycnometer, a thermometer, a viscometer, and a digital balance. The materials used were acetone, aquadest, phenolphthalein (PP) indicator, carbon with a particle size of 70 mesh, methyl acetate p.a. (Sigma Aldrich, 99%), potassium hydroxide (KOH), waste cooking oil, and cajuput oil.

Carbon Activation

Prior to use, the carbon was activated by weighing 5 g and soaking it in 100 mL of 2 M KOH solution for 24 h. The carbon was then filtered, washed with distilled water until neutral pH was achieved, and dried in an oven at 105°C for 30 min.

Pre-treatment of Waste Cooking Oil

Waste cooking oil was filtered to remove solid impurities and then mixed with 10 wt% activated carbon based on the oil mass. The oil was heated at 105°C for 10–15 min to reduce moisture content, while the activated carbon adsorbed impurities. The waste cooking oil was subsequently analyzed for acid value and moisture content.

Interesterification of Waste Cooking Oil Using Cajuput Oil Biocatalyst

Waste cooking oil (200 g) was weighed, and the oil-to-methyl acetate molar ratio was set at 1:6. Cajuput oil biocatalyst concentrations of 0.5%, 1.0%, and 1.5% (w/w, based on oil weight) were applied. The oil was placed in a three-neck round-bottom flask reactor and heated to the reaction temperature of 60°C. After reaching the desired temperature, methyl acetate and cajuput oil biocatalyst were added. The reactor was equipped with a reflux condenser, the temperature was maintained at 60°C, and stirring was carried out at 300 rpm using a magnetic stirrer. Reaction time was counted according to the predetermined variations of 20, 40, 60, 80, and 100 min. After completion of the reaction, 50 g of product sample was taken for each reaction time and cooled to room temperature.

Product and Residual Separation

The reaction samples were subjected to distillation at 60°C to separate unreacted methyl acetate from the reaction mixture. Distillation was continued until no further methyl acetate distillate was observed. The distillation residue, considered as the product, was weighed to determine the crude yield and analyzed for physical properties, including density, acid value, and viscosity. The product obtained under optimum conditions was further analyzed for FAME (Fatty Acid Methyl Ester) concentration using gas chromatography (GC). Crude yield (%) was calculated using Equation (1) (Daryono, Widi, et al., 2025).

$$Crude\ yield\ (\%) = \frac{Weight\ of\ product}{Weight\ of\ sample}\ x\ 100\% \quad (1)$$

Moisture Content Analysis of Waste Cooking Oil

A 10 g sample of waste cooking oil was weighed in a clean, dry porcelain dish to obtain the initial mass. The dish containing the oil was heated in an oven at 105°C for 20 min. After heating, the dish was removed and cooled in a desiccator to room temperature, then weighed again to obtain the final mass. All weighing results were recorded. If the moisture content was below 0.6%, the oil was deemed suitable for the interesterification reaction. Moisture content (%) was calculated using Equation (2).

$$Moisture\ content\ (\%) = \frac{Initial\ weight - final\ weight}{Initial\ weight}\ x\ 100\% \quad (2)$$

Acid Value Test

A 5 g sample was placed in an Erlenmeyer flask and dissolved in 20 mL of acetone, followed by the addition of three drops of phenolphthalein (PP) indicator. The sample was titrated with 0.1 N KOH until a pink color appeared and persisted for approximately 15 s. The volume of titrant used was recorded. The acid value (mg KOH/g) was calculated using Equation (3) (Shao & Agblevor, 2015).

$$Acid\ value\ (mg\frac{KOH}{g}) = \frac{56.1\ x\ volume\ KOH\ x\ N\ KOH}{Weight\ of\ sample} \quad (3)$$

Density Test

An empty 5 mL pycnometer was weighed, then filled with the sample and heated in a water bath to 40°C before being weighed again. Density (g/mL) was calculated using Equation (4) (Ansori et al., 2025).

$$Density\ (\frac{g}{mL}) = \frac{Weight\ of\ sample}{Volume\ of\ pycnometer} \quad (4)$$

Viscosity Test

The sample was heated in a water bath to 40°C and then introduced into an Ostwald viscometer, ensuring that no air bubbles were present. The liquid was drawn above the upper mark using a rubber bulb, which was then released while simultaneously starting a stopwatch. The time was recorded when the liquid passed the lower mark. Measurements were performed in triplicate. Viscosity was calculated using Equation (5) (Sufin & Daryono, 2024).

$$\frac{\eta_1}{\eta_2} = \frac{\rho_1\ x\ t_1}{\rho_2\ x\ t_2} \quad (5)$$

Where: η_1 is the sample viscosity, η_2 is the viscosity of distilled water, ρ_1 is the sample density, ρ_2 is the density of distilled water, t_1 is the flow time of the sample (s), and t_2 is the flow time of distilled water (s).

Optimization Criteria Used

Optimized Research Variables:

Concentration of cajuput oil biocatalyst : 0.5%, 1% and 1.50%

Reaction time : 20, 40, 60, 80 and 100 minutes

The selection of cajuput oil biocatalyst concentrations (0.5%, 1%, and 1.5%) was based on previous research that employed a biocatalyst concentration of 0.75% as a fixed variable (Daryono & Dewi, 2022). The researchers aimed to determine whether biocatalyst concentrations below and above 0.75% influence the resulting yield. The selection of reaction times ranging from 20 to 100 minutes was also based on prior research, which reported the highest crude yield of 65.88% at a reaction time of 75 minutes, indicating that optimal conditions had not yet been achieved (Daryono & Dewi, 2022). The researchers therefore sought to investigate whether reaction times below and above 75 minutes affect the resulting yield.

Optimized Result Response:

The crude yield (%), acid value (mg KOH/g), density (g/mL) and viscosity (cSt) from the result analysis for each research variable are shown in Table 1 below.

Table 1. Research Result Data Design

Concentration of Biocatalyst (%)	Reaction Time (minutes)	Crude Yield (%)	Acid Value (mg KOH/g)	Density (g/mL	Viscosity (cSt)
0.5	20				
	40				
	60				
	80				

1	100 20 40 60 80 100
1.5	20 40 60 80 100

RESULTS AND DISCUSSION

Interesterification of Waste Cooking Oil Using Cajuput Oil Biocatalyst

The initial analysis of waste cooking oil after pretreatment showed a moisture content of 0.062% and an acid value of 1.122 mg KOH/g; therefore, the oil was suitable for direct interesterification. Based on the calculations, crude yield data were obtained and are presented in Figure 1.

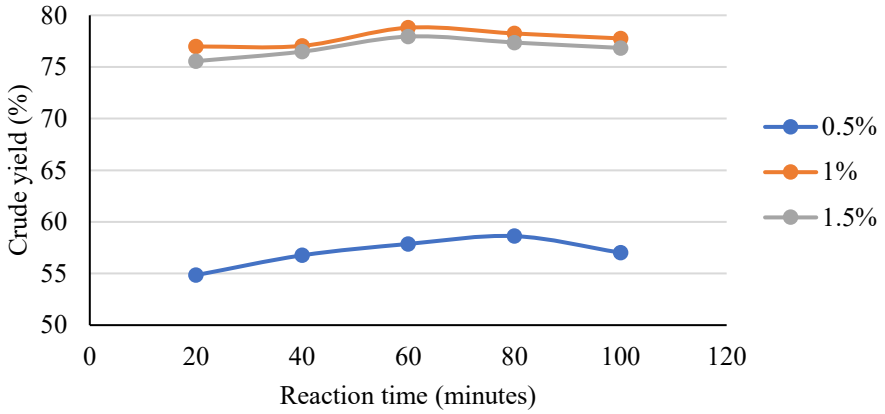

Figure 1. Effect of biocatalyst concentration and reaction time on crude yield

Figure 1 shows that the highest crude yield was achieved at a reaction time of 60 min with a biocatalyst concentration of 1%, reaching 78.80%, while the lowest crude yield was obtained at a reaction time of 20 min with a biocatalyst concentration of 0.5%, amounting to 54.84%. The low crude yield at shorter reaction times indicates that triglyceride conversion to esters had not yet proceeded optimally due to insufficient effective molecular collisions (Turnip et al., 2017). As the reaction time increased, crude yield also increased because more FAME was formed, allowing the interesterification efficiency to reach an optimum at approximately 60-80 min (Muarif et al., 2024).

At a biocatalyst concentration of 0.5%, optimum conditions were achieved at a reaction time of 80 min with a crude yield of 58.62%. At 1.5% biocatalyst concentration, the optimum was obtained at 60 min with a crude yield of 77.94%. Overall, a reaction time of 60 min represents the optimum point, as most triglycerides had been converted to biodiesel. Beyond this point, crude yield did not increase, indicating that the reaction had reached equilibrium. Excessive reaction times may induce reverse reactions and side reactions, leading to a decrease in crude yield (Eevera et al., 2009). These results indicate that a biocatalyst concentration of 1% is the most effective for enhancing reaction yield. Thus, the optimum condition in this study was achieved at a cajuput oil biocatalyst concentration of 1% and a reaction time of 60 min.

The results showed that a crude yield of 78.8% was obtained using 1% cajuput oil biocatalyst and a reaction time of 60 minutes, which is superior to previous research that reported a crude yield of 65.88% using a 0.75% biocatalyst and a reaction time of 75 minutes. This

improvement is attributed to the 1% cajuput oil biocatalyst containing a sufficient amount of the aromatic compound 1,8-cineole, which more effectively converts triglycerides and methyl acetate into methyl esters until the optimal reaction time is reached, compared to the 0.75% biocatalyst concentration.

Acid Value Test

The calculated acid value data are presented in Figure 2.

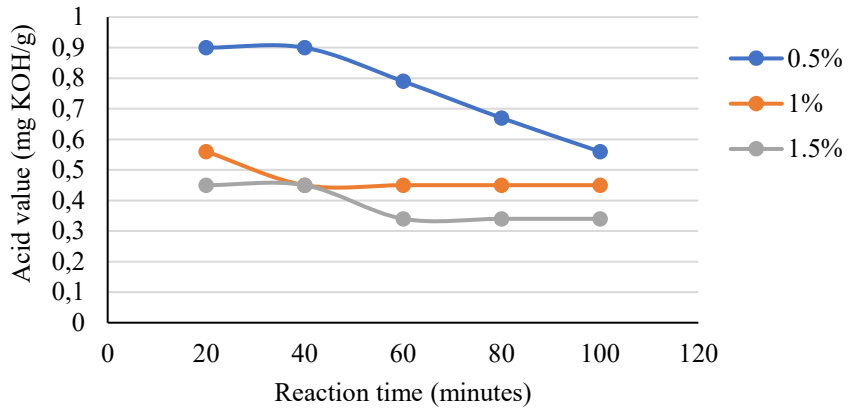

Figure 2. Effect of biocatalyst concentration and reaction time on acid value

Figure 2 shows that the highest acid value occurred at reaction times of 20 and 40 min with a biocatalyst concentration of 0.5%, reaching 0.90 mg KOH/g, whereas the lowest acid value was observed at reaction times of 60, 80, and 100 min with a biocatalyst concentration of 1.5%, reaching 0.34 mg KOH/g. The acid value tended to decrease with increasing reaction time and biocatalyst concentration, indicating a more effective interesterification process in which triglycerides were increasingly converted into fatty acid esters (Daryono & Dewi, 2022). A higher biocatalyst concentration also accelerated the decrease in acid value due to the greater availability of active sites, enhancing triglyceride conversion (Sativa et al., 2024).

Compared with the maximum acid value specified by SNI 7182:2015 (≤ 0.5 mg KOH/g), several operating conditions in this study met the standard, particularly at biocatalyst concentrations of 1% and 1.5% with reaction times longer than 20 min. These conditions produced biodiesel with improved stability, lower triglyceride content, and compliance with national standards.

Density Test

Density data obtained from calculations are shown in Figure 3.

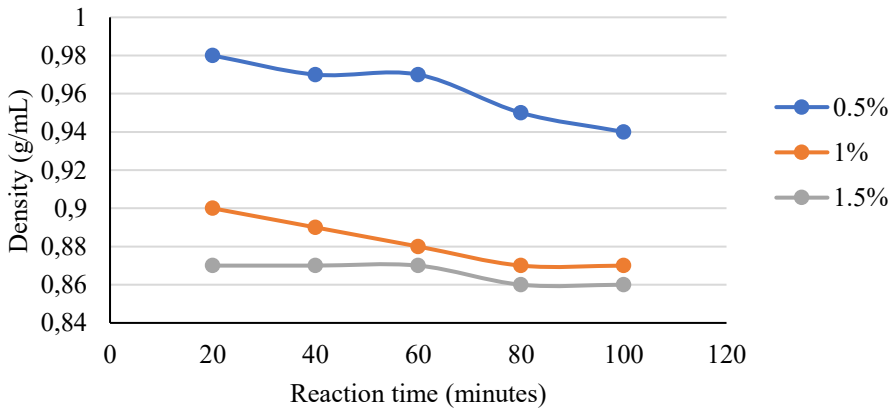

Figure 3. Effect of biocatalyst concentration and reaction time on density

Figure 3 indicates that the highest density was observed at a reaction time of 20 min with a biocatalyst concentration of 0.5% (0.98 g/mL), while the lowest density was obtained at 100 min with a biocatalyst concentration of 1.5% (0.86 g/mL). The decrease in density reflects improved conversion of triglycerides into fatty acid esters, resulting in biodiesel with simpler molecular structures and lower density than the original oil (Casas et al., 2011). Increasing biocatalyst concentration also accelerated the reaction, leading to a higher ester content and density values closer to biodiesel standards (Youssef et al., 2024).

According to SNI 7182:2015, which specifies a biodiesel density range of 0.85–0.89 g/mL, samples with a biocatalyst concentration of 1% at reaction times of 40–100 min and 1.5% at 20–100 min complied with the standard. This confirms that longer reaction times and higher biocatalyst concentrations are effective in producing biodiesel with suitable density characteristics (Marlina et al., 2020).

Viscosity Test

Viscosity data are presented in Figure 4.

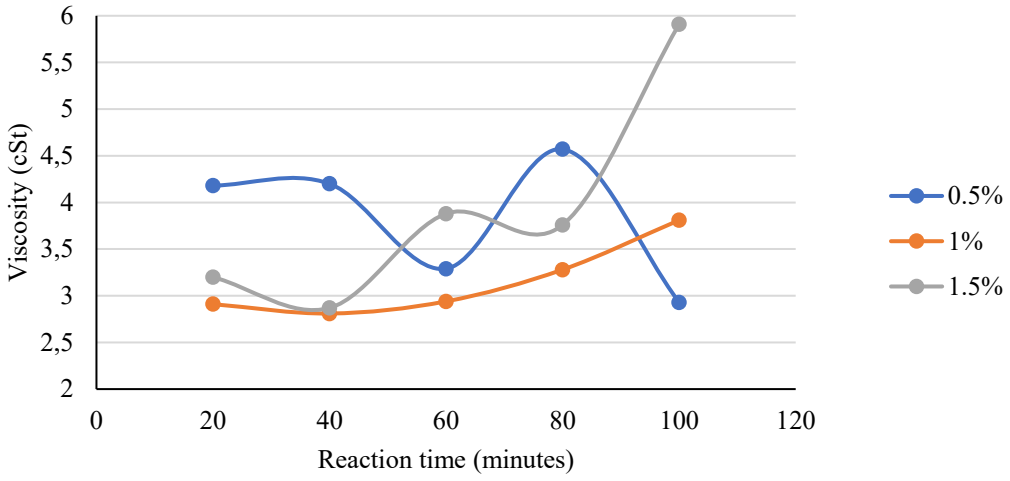

Figure 4. Effect of biocatalyst concentration and reaction time on viscosity

Figure 4 shows that the highest viscosity was obtained at a reaction time of 100 min with a biocatalyst concentration of 1.5% (5.91 cSt), while the lowest viscosity was observed at 40 min with a biocatalyst concentration of 1% (2.81 cSt). These differences reflect the extent of triglyceride conversion to esters. At shorter reaction times, incomplete conversion results in heavier molecules that increase viscosity (Turnip et al., 2017). With longer reaction times, viscosity generally decreases due to increased FAME formation and lighter molecular structures (Muarif et al., 2024).

Fluctuations in viscosity are attributed to the reversible nature of interesterification reactions, particularly in the waste cooking oil–methyl acetate system. Beyond the optimum point, reverse reactions may occur, reforming heavier glyceride compounds and increasing viscosity, as observed at 1.5% biocatalyst concentration and 100 min reaction time (Hussein & Idris, 2024). Although higher biocatalyst concentrations generally promote ester formation and reduce viscosity, excessive catalyst levels combined with prolonged reaction times may lead to the formation of residues or side products that increase viscosity (Orchidantya et al., 2023). The presence of aromatic compounds from cajuput oil in biodiesel weakens van der Waals forces, resulting in a decrease in viscosity (Marlina et al., 2020; Wardoyo et al., 2021)

Compared with SNI 7182:2015, which specifies a viscosity range of 2.3–6.0 cSt at 40 °C, all samples in this study fell within the acceptable range, indicating that cajuput oil biocatalyst under optimum conditions can produce biodiesel with suitable flow properties.

GC Analysis Results

The GC chromatogram obtained under optimum conditions (1% biocatalyst concentration and 60 min reaction time) is shown in Figure 5, while the identified FAME components and their concentrations are listed in Table 1.

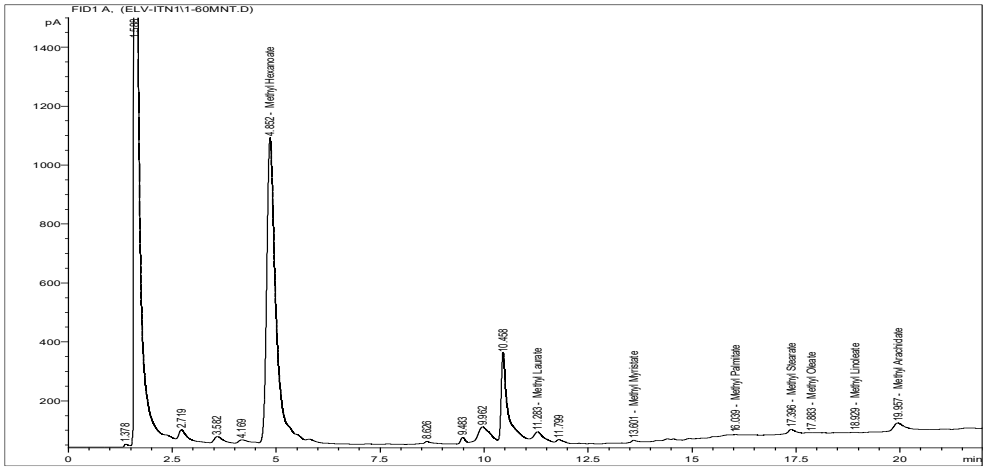

Figure 5. GC chromatogram of FAME under optimum process conditions

Table 1. FAME components identified by GC under optimum conditions

Residence Time (min)	Component of FAME	Concentration (mg/L)
4.852	Methyl Hexanoate	13,731.90
11.283	Methyl Laurate	708.98
13.601	Methyl Miristate	213.05
16.039	Methyl Palmitate	710.37
17.396	Methyl Stearate	1,446.96
17.883	Methyl Oleate	303.09
18.929	Methyl Linoleate	464.41
19.957	Methyl Arachidate	1,827.71
	Total	19,406.50

Based on GC analysis, both long-chain and short-chain methyl esters were identified in the biodiesel sample. Long-chain methyl esters included methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and methyl arachidate. The high concentration of methyl hexanoate indicates that interesterification with methyl acetate under these conditions favored the formation of shorter-chain esters. This may occur because methyl acetate, as an acyl donor, reacts more rapidly with free fatty acids and certain glyceride structures, promoting the formation of shorter-chain esters under the applied reactant ratio and catalyst activity (Sandra et al., 2021).

The dominance of FAME peaks confirms that the interesterification process proceeded effectively, converting most fatty acids into esters and producing biodiesel with high purity (Ramadhani et al., 2025). Saturated methyl esters such as methyl palmitate and methyl stearate contribute to improved oxidative stability due to their resistance to thermal degradation, while unsaturated methyl esters such as methyl oleate and methyl linoleate enhance flow properties and reduce viscosity. This balanced composition of saturated and unsaturated FAME indicates favorable biodiesel characteristics in terms of both stability and performance (Mulminin et al., 2023).

CONCLUSION

Based on the results of this study on biodiesel production from waste cooking oil through an interesterification process using cajuput oil biocatalyst at a reaction temperature of 60°C, an

oil-to-methyl acetate molar ratio of 1:6, and a stirring speed of 300 rpm, it can be concluded that variations in biocatalyst concentration and reaction time significantly affect the acid value, density, viscosity, crude yield, and FAME composition of the biodiesel produced. The optimum conditions were achieved at a cajuput oil biocatalyst concentration of 1% and a reaction time of 60 min, resulting in a crude yield of 78.80%, an acid value of 0.45 mg KOH/g, a density of 0.88 g/mL, and a viscosity of 2.94 cSt, all of which comply with SNI 7182:2015, as well as a FAME concentration of 19,406.50 mg/L. The results of this study are expected to be applicable at the pilot-plant scale, particularly for a biodiesel production process that utilizes waste feedstocks and involves short processing steps, thereby reducing production costs and enabling competitiveness with diesel fuel prices.

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