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Production of Biodiesel Using Enzymatic Esterification of Multi-Feedstock Oils

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Abstract. Biodiesel is produced from oils esterified with alcohol into glycerol and water. Vegetable edible oil raw materials are the main consideration in biodiesel production. This study used three types of oil, namely palm oil (PO), waste frying oil (WFO), soybean oil (SO), and corn oil (CO), with the catalyst of the enzyme lipase. The price of WFO as raw materials is low, although it must be controlled acid and water levels. In the research run, the oil mixture consists of two types of oils mixed with a certain composition and the addition of certain lipase enzymes. The research resulted that the yield produced by multi-feedstock biodiesel with free fatty acid (FFA) < 2 was 89.7%, 89.03%, and 86.11% higher than the sample with FFA > 2 at 79.54%, 74.66%, and 73.33%, respectively. The minimum density produced is a mixture of WFO with CO of 861.1 kg/m^3. The largest viscosity produced is a mixture of WFO with SO of 18.03 mm^2/s. Mixing raw materials can lower the number of iodine multi-feedstock biodiesel. The number of acids produced by multi-feedstock biodiesel exceeds ASTM standards. The total glycerol produced by multi-feedstock biodiesel varies, whereby a multi-feedstock blend of PO can lower total glycerol. In contrast, a multi-feedstock blend of WFO tends to produce high total glycerol.

Keywords: Biodiesel; Free Fatty Acid; Multi Feedstock; Lipase; Waste Frying Oil

INTRODUCTION

Biodiesel has emerged as a viable alternative energy source (Abomohra et al., 2020) in response to the depletion of petroleum reserves (Aparamarta et al., 2020). Biodiesel, or fatty acid methyl ester (FAME), is produced primarily from edible oils such as soybean palm and rapeseed oil. Methanol is commonly used as an acyl-acceptor in biodiesel production (Chang et al., 2021). However, the expensive cost of edible oils as basic materials for biodiesel presents economic challenges. In addition, the price of these oils is heavily influenced by market factors, which can affect their availability for human consumption (low or high oil prices) (Dutta et al., 2021).

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The expensive cost of basic materials is the greatest hindrance to biodiesel production. Food-grade oils as the primary raw material hinder large-scale production substantially compared to conventional fossil fuel-based diesel (Rodrigues et al., 2021). It is estimated that 70 to 95% of the total cost of biodiesel production is attributable to the cost of basic materials (Farooq et al., 2015).

There have been efforts to address the basic material costs associated with biodiesel production. Elgharbawy et al. (2002) suggest utilizing refuse heating oil, considered non-useful. Using residual frying oil as a substrate serves multiple purposes: it reduces the high cost of raw materials, improves the economic viability of the production process, and prevents competition with edible oils required for human consumption. However, the production of biodiesel from used cooking oil presents some obstacles. Waste frying oil has a higher concentration of free fatty acids (FFAs) and water, which can hinder biodiesel production (Pourzolfaghar et al., 2016). Consequently, biodiesel production from used cooking oil must be carefully considered.

Biodiesel production has been extensively researched using various feedstocks, including palm oil, soybean oil, corn oil, and waste frying oil. However, there are still research gaps and areas that require further exploration. Palm oil is widely used due to its high oil yield; converting it into biodiesel faces challenges in terms of reaction efficiency and catalyst optimization (Mukhtar et al., 2022). Soybean oil is another popular feedstock, but its availability and price volatility present challenges. Biodiesel production from soybean oil involves multiple steps, which require energy, chemicals, and specialized equipment, contributing to the overall production cost (Laskar et al., 2020). Corn oil, a byproduct of the corn milling industry, has gained attention as a potential feedstock. However, extracting oil from corn kernels is a complex process that affects yield and cost-effectiveness (Basyouny et al., 2021). Waste frying oil, from used cooking oils, offers an opportunity for biodiesel production, addressing waste management and providing an economically viable and sustainable feedstock option. However, waste cooking oil can contain impurities and higher levels of free fatty acids, impacting biodiesel quality & yield (Chen et al., 2021). Exploring novel approaches for collecting, purifying, and converting waste frying oil into high-quality biodiesel is crucial.

Transesterification and esterification are two of the most prevalent biodiesel synthesis processes. Transesterification is generally a reaction between oil-based triglycerides and short-chain alcohol such as methanol or ethanol. Utilizing a catalyst to accelerate the reaction, fatty esters (the biodiesel product) and glycerol are produced. The readily available basic materials for biodiesel production include vegetable oils, animal lipids, refuse cooking oil, and even algae (Miraculas, 2018). Enzymes are frequently used as catalysts in biodiesel synthesis in large-scale industrial production, such as in the pharmaceutical, food, and specialty chemical industries (Chapman et al., 2018). Enzymes offer several benefits in industrial applications, including high productivity, low costs, and the suppression of unwanted byproducts (Maldonado, 2016). Their ubiquitous use can be attributed to characteristics such as high stereoselectivity, simplicity of production, and substrate specificity (Davids et al., 2013). Enzymes have limitations, such as sensitivity to reaction conditions and minimal operational stability.

These characteristics hinder the large-scale application of certain enzymatic processes. Cipolatti et al. (2017) state that immobilization techniques, selectivity improvements, and inhibitor resistance enhancement have been utilized to surmount these limitations. These strategies are designed to optimize enzymatic reactions and make them more suitable for efficient and robust biodiesel production on a large scale.

Lipases are hydrolases of the triacylglycerol ester and are typically used as biocatalysts due to their high performance of activity and stability due to the presence of organic solvents, as they can set up for various types of substrates, including esters of fatty acids, triacylglycerides, synthetic and natural oils, lipids, etc. (Bezerra et al., 2017). Yeast and filament-type fungi and some genera are the main producers of lipases and almost 50% of the lipase commercial volume, namely: *Candida, Aspergillus, Geotrichum, Fusarium, Pseudomonas, Mucor, Rhizopus, Penicillium* and *Rhodotorula* (Maldonado, 2016). Utilizing lipase enzymes as catalysts in biodiesel production has acquired popularity due to their ability to produce high-quality biodiesel at low operating temperatures and a straightforward downstream process. Enzymes offer adaptability and make it possible to obtain high biodiesel yields under optimal conditions (Mandari and Devarai, 2021). Biodiesel is produced by transesterification, which involves the reaction of lipids and vegetable oils with methanol. Using enzyme as biocatalysts, this process has two forms of biodiesel that function as biofuels: fatty acid methyl ester (FAME) and fatty acid ethyl ester (FAEE). One type of lipase enzyme used in the transesterification process is Amobil P. Cepacia, Novozym 435, and Lipozyme IM.

In this study, multi-feedstock biodiesel will be produced with four oil feedstocks whose proportions will vary dependent on the volume of each composition. The oil mixture will be converted into biodiesel via transesterification with the aid of the *Pichia pastoris* lipase enzyme as a catalyst. This study examines the effect of differences in the composition of vegetable oil-fatty acids on the purity of biodiesel produced by transesterification with methanol and the *Pichia pastoris* lipase enzyme as a catalyst.

MATERIALS AND METHOD

Materials

The experiment employed three kinds of cookery oils, namely palm oil (PO), soybean oil (SO), and maize oil (CO), all of which were purchased from Indonesian stores. In addition, waste frying oil (WFO) was gathered from a restaurant near Semarang, Central Java, Indonesia. These four oils were chosen due to their high triglyceride content. In the experiments, methanol obtained from chemical stores in Semarang, Central Java, Indonesia, served as the alcohol. In each iteration, the volume ratio of methanol to oil was maintained at 1:8, with a total volume of 250 mL (Table 1). To coordinate the investigation, a matrix of the experimental design (Table 2) was constructed. This matrix defined the specific conditions and parameters to be examined during the research procedure.

This research involved two distinct GCMS analyses. The initial study centered on a singular palm oil feedstock with a free fatty acid (FFA) content of less than 2. The second analysis was conducted on a sample of a mélange of waste fryer and soybean oil (multi-feedstock) with an FFA content greater than 2. The composition of the biodiesel

derived from palm oil is shown in Table 3 based on the results of the GCMS analysis performed on the palm oil sample. Table 4 displays the results of the GCMS analysis of the residual frying oil and soybean oil mixture and its fatty acid contents.

Table 1. Oil composition of raw materials

Type of fatty acids	Palm oil	Waste frying oil	Soybean oil	Corn oil
Lauric (C12:0)	< 1,2	1	0 – 0,1%	
Myristic (C14:0)	0,5 – 5,9	1,1 – 2,5		0,1
Palmitic (C16:0)	32 – 59	40 – 46	7 – 10%	8 – 12
Palmitoleic (C16:1)	< 0,6	-		0,1
Stearic (C18:0)	1,5 – 8	3,6 – 4,7	2 – 5%	2,5 – 4,5
Oleic (C18:1)	27 – 52	30 - 45	11 – 60%	19 – 49
Linoleic (C18:2)	5,0 – 1,4	7 – 11	15 – 64%	34 – 62
Linolenic (C18:3)	< 1,5	1	1 – 12%	1,2
Arachidonic acid			1,5%	
Arachidic acid			0,2 – 1%	

Table 2. Experiment design

Run	Oil composition	Volume ratio	Addition of lipase enzyme (% vol)	Enzyme volume (mL)	Methanol volume (mL)	Mixed oil volume (mL)
1.	PO : WFO	1 : 1	1%	2.5	27.5	220.0
2.	PO : WFO	1 : 1	2%	5.0	27.3	217.7
3.	PO : WFO	1 : 1	3%	7.5	26.9	215.6
4.	PO : SO	1 : 2	1%	2.5	27.5	220.0
5.	PO : SO	1 : 2	2%	5.0	27.3	217.7
6.	PO : SO	1 : 2	3%	7.5	26.9	215.6
7.	PO : CO	1 : 2	1%	2.5	27.5	220.0
8.	PO : CO	1 : 2	2%	5.0	27.3	217.7
9.	PO : CO	1 : 2	3%	7.5	26.9	215.6
10.	WFO : SO	2 : 1	1%	2.5	27.5	220.0
11.	WFO : SO	2 : 1	2%	5.0	27.3	217.7
12.	WFO : SO	2 : 1	3%	7.5	26.9	215.6
13.	WFO : CO	2 : 1	1%	2.5	27.5	220.0
14.	WFO : CO	2 : 1	2%	5.0	27.3	217.7
15.	WFO : CO	2 : 1	3%	7.5	26.9	215.6
16.	SO : CO	1 : 1	1%	2.5	27.5	220.0
17.	SO : CO	1 : 1	2%	5.0	27.3	217.7
18.	SO : CO	1 : 1	3%	7.5	26.9	215.6

Table 3. GCMS results of palm oil

Acid content	Percentage (%)
Lauric acid (C12:0)	0.13
Myristic acid (C14:0)	1.02
Palmitic acid (C16:0)	37.75
Linoleic acid (C18:2)	11.65
Oleic acid (C18:1)	28.42
Stearic acid (C18:0)	7.79
Palmitic acid beta-monoglyceride (C19)	3.98
2-mono-beta-Monolinoleic (C12)	9.26

Table 4. GCMS results of sample mixture of waste frying and soybean oils

No	Composition	Content (%)
1	Lauric acid	0.09
2	Myristic acid	0.50
3	Pentadecanoic acid	0.04
4	Palmitic acid	22.73
5	Palmitoleic acid	0.24
6	Heptadecanoic acid	0.05
7	Cis-10-Heptadecanoic Acid	0.03
8	Stearic acid	3.24
9	Elaidic acid	0.08
10	Oleic acid	33.30
11	Linoleic acid	26.15
12	Arachidic acid	0.26
13	v-Linolenic acid	0.12
14	Cis-11-Eicosenoic acid	0.14
15	Linolenic acid	2.25
16	Cis-11,14-Eicosedienoic acid	0.03
17	Behenic acid	0.13
18	Arachidonic acid	0.02
19	Tricosanoic acid	0.02
20	Lignoceric acid	0.07
21	Cis-5,8,11,14,17-Eicosapentaenoic acid	0.02
22	Nervonic acid	0.05
23	Cis-4,7,10,13,16,19-Docosahexaenoic acid	0.05

Experimental Equipment

Equipment used for transesterification is carried out in a flask with a triple neck flat-bottom equipped with reverse cooling, temperature control, water bath, and stirrer. The experiments were conducted at a temperature of 35 °C, a reaction time of 20 hours, and a stirring speed of 200 rpm.

Experimental Procedure

Determine the concentration of free fatty acids first (ASTM D 664) as follows: (1). Weigh the sample oil mixture as much as 2.5 g and put it in Erlenmeyer, (2). Add 25 ml of ethanol 95% and 25 ml of chloroform to the Erlenmeyer that already contains the sample, then add the PP indicator as much as 2 drops into the Erlenmeyer (3). The sample is then iterated with NaOH 0.05 N until the solution turns pink and does not disappear for 30 seconds (4), Recording titrant volume requirements.

Prepare the material for the experiment according to the design of experiments as in Table 2. Insert the oils, methanol, and the enzyme into a triple neck flat-bottom flask of 350 mL, and shake gently to mix the materials with a magnetic stirrer. Put the flask into the water bath, and set up the operating condition at 35°C, stirring speed of 200 rpm, and the operating time for reaction of 20 hours. After the operation time, the result of transesterification oil was transferred into the glass. The mixture was transferred to the centrifuge glass and separated by a centrifuge glass at 1500 rpm in 15 minutes. The mixture will result in three layers based on the difference in density. The product of methyl ester is on the top layer, separating the top layer from the layers to remove the remaining methanol from the product, the methyl ester is heated using the oven to a temperature of 1100 °C, and then the

remaining product was separated and put in the glass. Weigh the product of biodiesel and prepare to analyze for biodiesel parameters of density at 400 °C (ASTM D 1298), kinematic viscosity at 400C (ASTM D 445), Cetane number, Iodine number (AOCS Cd 1-25), and yields.

RESULTS AND DISCUSSION

The FFA Value of Multi-Feedstocks

The free fatty acid (FFA) level variation between various multi-feedstocks significantly impacts biodiesel production yield. Table 5 measures FFA levels categorized as FFA > 2 and FFA < 2.

Table 5. Results of the FFA of the various composition of the oils

Run	Oil composition	Volume ratio	FFA (%)
1.	PO : WFO	1 : 1	> 2
2.	PO : WFO	1 : 1	> 2
3.	PO : WFO	1 : 1	> 2
4.	PO : SO	1 : 2	< 2
5.	PO : SO	1 : 2	< 2
6.	PO : SO	1 : 2	< 2
7.	PO : CO	1 : 2	< 2
8.	PO : CO	1 : 2	< 2
9.	PO : CO	1 : 2	< 2
10.	WFO : SO	2 : 1	> 2
11.	WFO : SO	2 : 1	> 2
12.	WFO : SO	2 : 1	> 2
13.	WFO : CO	2 : 1	> 2
14.	WFO : CO	2 : 1	> 2
15.	WFO : CO	2 : 1	> 2
16.	SO : CO	1 : 1	< 2
17.	SO : CO	1 : 1	< 2
18.	SO : CO	1 : 1	< 2

Elma et al. (2016) conducted a study that revealed that oils with a higher FFA content have lower quality for biodiesel production. In addition to FFA content, oleic acid in each oil contributes to decreased yields caused by oxidation. Due to its low activation energy, oleic acid is particularly susceptible to oxidation reactions (Wang et al., 2021). WFO has the maximum oleic acid content, ranging from 30 to 45%, compared to PO, SO, and CO, which contain 27%, 19%, and 11% oleic acid, respectively. Significant levels of oleic acid prevent the formation of methyl esters, essential for biodiesel production. In addition, volatile fatty acids are present and are used by lipase enzymes as carbon sources, contributing directly to the elongation of fatty acid chains by microorganisms.

Effect of The Composition of Multi Feedstocks to The Density for Various Concentrations of Enzyme Addition

Palmitic acid (a saturated oil), oleic acid, and linoleic acid (unsaturated oils) are the three primary components of the basic material used for biodiesel production. The selection of raw materials abundant in unsaturated (e.g., oleic and linoleic acids) or saturated (e.g., palm oil) fatty acids affects the performance of biodiesel properties. This option can either increase or decrease the biodiesel's frigid flow properties. When unsaturated fatty acids are present, such as in neem seed pyrolysis oil (982 kg/m^3), the product density of biodiesel tends to be greater. Additionally, unsaturated fatty acids can reduce biodiesel's viscosity, influencing its flashpoint. The density, viscosity, and carbon residue of biodiesel derived from non-edible plant oils are high. Combined with incomplete combustion, these properties can increase particulate and NOx emissions from biodiesel.

Enzymes are regarded as advantageous transesterification catalysts due to their

capacity to eradicate the difficulties associated with the purification, cleansing, saponification, and neutralization processes. Among their most promising characteristics are their suitability for high free fatty acid (FFA) feedstocks, absence of by-product generation, operation under moderate reaction conditions, and higher oil-to-biodiesel conversion rates. However, enzymes have several disadvantages, including a high price tag, a prolonged reaction time, and a low utilization rate (Adhani et al., 2016). For biodiesel, ASTM density standard of 860 – 900 kg/m3. The density of PO, WFO, SO, and CO is 914 kg/m^3, 897 kg/m^3, 922 kg/m^3, and 925 kg/m3 (SNI). The density of raw material is between 897 to 925 kg/m^3. Biodiesel is affected by many factors, such as the profile of methyl esters generated, the type of source material used, and the biodiesel production procedure (Pratas et al., 2011). In addition, the composition of fatty acids in the source materials and the purity of the biodiesel determine the density of biodiesel. Since the densities of methanol and oil are comparable to that of the produced biodiesel, numerous studies have observed that the density of biodiesel remains relatively stable (Encinar et al., 2005). Before the reaction occurs, the density of most mixed oil compositions is typically greater than the standard range. The addition of enzyme resulted that for 1% addition density of around 900.6 – 912.22 kg/m^3, 2% of 861.1 – 914.3 kg/m^3, and 3% of 877.1 – 904.3 kg/m^3 (Fig. 1). Density of biodiesel of waste cooking oil with heterogeneous catalyst of 863 kg/m3 (Farooq et al., 2015), high free fatty acids through glycerolysis: 840 kg/m^3 (Elgharbawy et al., 2021). Adding 1% enzyme resulted in a greater density than the standard value. When only 2% of the data was considered, only the mixed oils WFO and SO and WFO and CO fell within the acceptable range, whereas the others exceeded it. However, with the addition of 3% enzyme, the density met the standard requirement. Enzymes serve as catalysts to speed up a chemical reaction by promoting adequate contact between the catalyst and reactants. The results indicated that a minimal % enzyme addition of 3% was necessary to convert the reactants into biodiesel (Monteiro et al., 2021). In addition, the results indicated that the concentration of free fatty acids (FFA) did no affect on the enzymatic reaction about the density parameter.

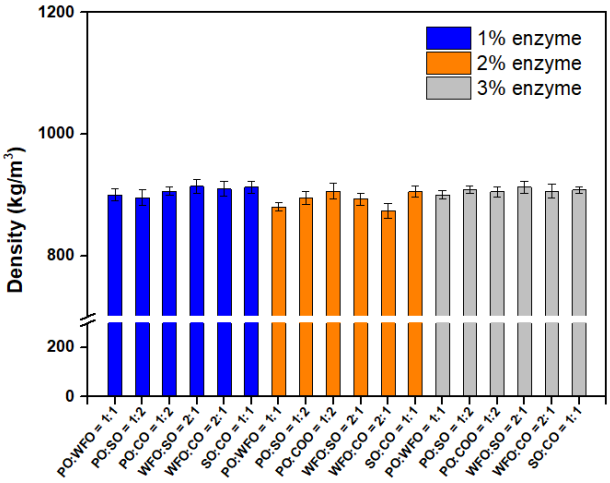

Fig. 1: Density of the various composition of multi feedstocks with addition of, 1, 2, and 3 % enzyme

Kinematic Viscosity

Fuel viscosity is essential in biodiesel combustion, particularly the purity of the fuel-air mixture. Extremely low or high viscosity cannot be tolerated for engine operation; low viscosity causes low penetration and combustion, emmiting black fumes. While fuel with a high viscosity or viscosity will penetrate the opposite injector wall, resulting in a frigid cylinder surface and low fuel combustion (Sahar et al., 2018). Based on ASTM D445, the quality

requirement of kinematic viscosity of biodiesel produced at 400 °C is 2.3-6.0 mm^2/s.

Figure 2 depicts the viscosity data obtained from varying enzyme additions (1%, 2%, and 3%) for diverse multi-feedstock compositions. All of the obtained viscosity data exceeded the standard value. The presence of palmitic, oleic, and linoleic oils in the raw materials affects the viscosity of biodiesel, with higher saturation levels resulting in viscosity values exceeding the standard (Athar & Zaidi, 2020). On the other hand, unsaturated fatty acids in the raw materials contribute to biodiesel with a higher density, thereby reducing viscosity (Cavalcante et al., 2021). Biodiesel comprises methyl or ethyl esters of fatty acids and is derived from hydrocarbons. While some biodiesel reaches the standard range and can be used directly in diesel engines, many oils do not meet the required properties, especially in viscosity and melting temperature, up to ten times the normal values (Monteiro et al., 2021). Viscosity is affected by the configuration of double bonds in the biodiesel structure, with cis double bonds resulting in reduced viscosity and trans double bonds resulting in higher viscosity. Additionally, the number of double bonds influences viscosity, with lubricants containing one double bond exhibiting greater viscosity than those containing two or three double bonds. Additionally, branching structures in the ester chain and hydroxyl groups in the molecule contribute to increased viscosity (Athar & Zaidi, 2020). The quantity of catalyst used also contributes to the higher viscosity of biodiesel compared to the standard value. An increase in catalyst quantity results in a decrease in biodiesel yield, an increase in viscosity, and an increase in detergent formation (Cavalcante et al., 2021).

A high viscosity negatively affects the lubrication of pumps and injectors in machinery. Due to its oxygen content, which possesses electronegative properties, biodiesel exhibits greater polarity than diesel fuel. In general, biodiesel has a greater viscosity than diesel fuel. Furthermore, the presence of ethyl esters in biodiesel results in a greater viscosity than methyl esters (Barabás & Todoruţ, 2011). There is an intriguing correlation between the viscosity and density of biodiesel, in which an increase in density corresponds to a decrease in viscosity (Mujtaba et al., 2021). However, when enzyme concentrations range between 1% and 3%, the resulting density decreases, increasing the viscosity of the biodiesel produced.

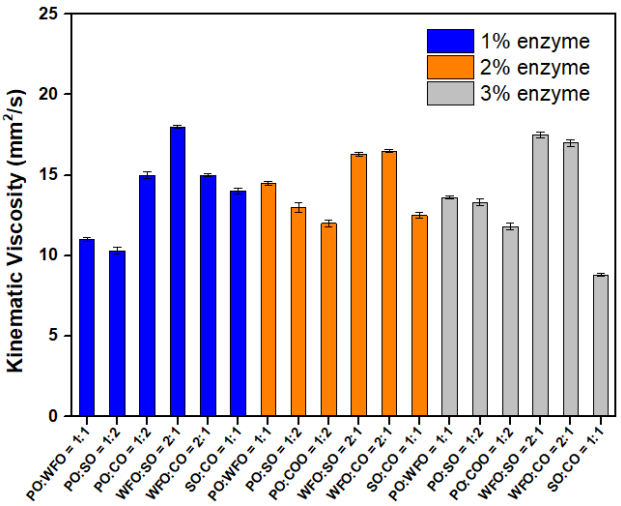

Fig. 2: Kinematic viscosity of the various composition of multi feedstocks with addition of, 1, 2, and 3 % enzyme

Effect of Enzyme Concentration on The Cetane Number

The Cetane number is a dimensionless parameter used to evaluate the fuel grade for compression ignition engines. It functions as the primary indicator during the compression

stage of the propellant ignition phase of the combustion process. The Cetane number defines the ignition characteristics of the fuel in detail. Several variables affect the Cetane number, including double bonds in fatty acid ester molecules (measured by the iodine number) and an increase in carbon atoms. Ethyl esters generally have higher Cetane numbers than methyl esters, whereas methyl and ethyl linoleic esters have lower Cetane numbers (Barabás & Todoruţ, 2011).

The structure of biodiesel-component fatty acids affects the Cetane number. The lower the Cetane number, the more double bonds and the shorter the carbon chain (Mujtaba et al., 2021). In addition to double bonds and short carbon chains, an increase in unsaturated degrees decreases the Cetane number (Lin and Wu, 2021). According to Omwoyo et al., (2023) the Cetane number decreased as chain length decreased and branching increased. In aromatic compounds, petrodiesel has a low Cetane number, but this number rises as the extent of the n-alkali side chain grows.

Due to increased deposits, imperfect combustion, and clanging, biodiesel's low Cetane number results in higher engine emissions (Atabani et al., 2012). According to Sakthivel et al. (2018), the Cetane number increases the degree of saturation and elongation of fatty acid chains. Biodiesel with a higher Cetane number contains more oxygen, resulting in greater combustion efficacy.

Multi feedstock biodiesel produced (Fig 3) has a Cetane number for various addition of enzymes of 1% (49.37-55.87), 2% (49.58-55.45), 3% (48.6-56.4). The Cetane numbers obtained in this study were higher than the conventional Cetane number (>47). The presence of FFA and the composition of fatty acids influence biodiesel quality. Biodiesel's Cetane number, flash point, cold flow properties, and oxidation stability are largely determined by the fatty acid composition of the feedstock oil. Long-chain fatty acids contribute to increased Cetane and saturation levels. The relationship between fatty acid composition and variations in biodiesel properties can be explained by the fact that extremely unsaturated components (such as grape seed oils, sunflower oils, and soybean oils) have low Cetane numbers on average. In contrast, saturated fatty acids in biodiesel (such as palmitic acid and stearic acid) produce extremely high Cetane concentrations (Thilakarathne et al., 2021).

The Cetane number indicated that compositions containing oleic and linoleic oils from soybean and corn oil had a lower Cetane number than compositions containing a greater proportion of palmitic oil (palm oil and residual frying oil). In contrast, adding 1, 2, or 3% *Pischia pastoris* enzyme as a catalyst has no significant effect on the Cetane number. The composition of oleic and linoleic oils has a lower Cetane number than stearic and palmitic oil.

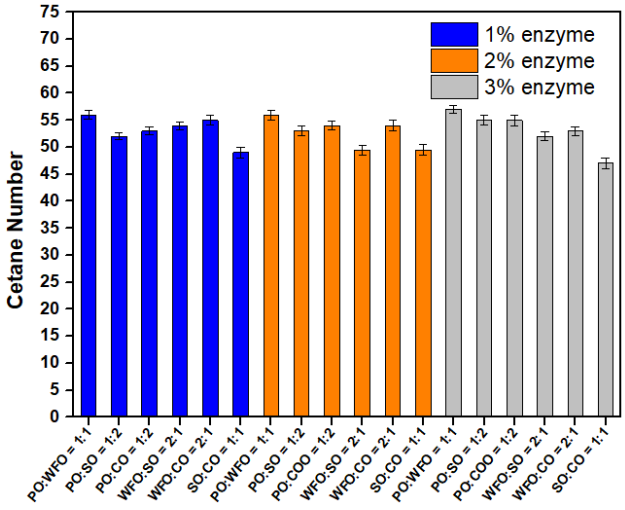

Fig. 3: Cetane number of the various composition of multi feedstocks with addition of, 1, 2, and 3 % enzyme

Effect of Enzyme Concentration on The Iodine Number

The iodine number is used to determine the level of saturation and oxidation tendency of fuel when exposed to air. However, the iodine number does not account for the precise locations of double bonds that undergo oxidation. The ASTM standard permits a maximum iodine number of 115 gr I_2/100 g. Except for the mélange of WFO and SO, the iodine content of multi-feedstock biodiesel generally meets ASTM requirements. The combination of WFO and SO produces iodine levels that exceed the ASTM standard. This is because combining oils changes fatty acids' composition in the frying oil, primarily composed of unsaturated fatty acids with double bonds. The presence of double bonds in fatty acids has an immediate effect on the iodine number. High iodine content biodiesel is undesirable because it tends to undergo polymerization, rendering it less stable. Notably, the iodine numbers produced by multi-feedstock biodiesel generally meet ASTM standards, except the WFO and SO mixture, which produces higher iodine numbers (as shown in Fig. 4) due to the altered composition of fatty acids present in the cooking oil, which is rich in unsaturated fatty acids with double bonds. Biodiesel with a high iodine content is preferable because it is more susceptible to polymerization and less durable (Adhani et al., 2016).

Effect of Enzyme Concentration on The Cetane Number

Enzymes are extensively utilized as catalysts in biodiesel production. They provide several benefits for the transesterification procedure. Enzymes eradicate the need for additional procedures such as product purification, rinsing, saponification, and neutralization, which is a significant advantage. Enzymes can be used with high free fatty acid (FFA) feedstocks, operate under moderate reaction conditions, and efficiently convert oil to biodiesel from a process standpoint. However, enzymes have some disadvantages, such as slower reaction times, greater costs, and limited application frequency. (Athar & Zaidi, 2020) Managing enzymes for commercial purposes presents challenges associated with recycling and stability maintenance. The catalyst concentration significantly impacts biodiesel yield. To promote the reaction at the desirable location, the catalyst must possess a high surface activity. The presence of more surface-active sites increases the yield of biodiesel produced (Cavalcante et al., 2021).

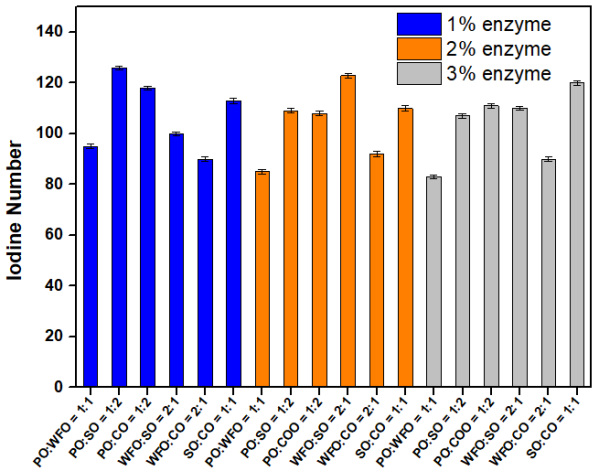

Fig. 4: Iodine number of the various composition of multi feedstocks with addition of, 1, 2, and 3 % enzyme

The oil-to-methanol mole ratio is the most important variable in the transesterification process. Ethanol, propanol, isopropyl alcohol, butanol, and pentanol can replace methanol. Due to its reduced commercial price than other alcohols, methanol is strongly suggested for biodiesel production. Unlike ethanol, methanol does

not form an azeotropic mixture with water that can be readily recycled from biodiesel products. Three moles of triglycerides react with one mole of methanol to generate three moles of methyl esters and one mole of glycerin, according to stoichiometry (Reddy et al., 2017).

The concentration of methanol is extremely crucial. Less or more methanol in the reaction system resulted in a low biodiesel yield. The transesterification process is reversible. If the ratio of oil to methanol is too low, product formation will not occur. Therefore, the transesterification reaction will shift toward reactants not producing biodiesel. This is the transesterification process' essence and the time when the reversible took place. Typically, reaction systems are designed with an excess of one of the reactants so that the reaction can be completed. While the ratio of oil to methanol is excessively high, methanol will overwhelm the catalyst's active sites. This decreases the possibility of catalyst-oil interaction. Separating glycerol from biodiesel is problematic when there is an excess of methanol. When there was an excess of methanol in the reaction, glycerol's density decreased, which hindered the separation of the excess methanol. When a higher oil-to-methanol ratio was utilized, these issues decreased biodiesel yield and increased methanol recovery costs (Sharma et al., 2018).

Raw waste cooking oil (WCO) material with methanol to oil ratio of 6:1, 0.7 wt.% of NaOH as catalyst, 90 minutes reaction and 500C reaction temperature, the conversion was 86%. Meanwhile, the production of biodiesel using coconut oil as raw material and NaOH as a catalyst, under the optimal condition, 0.5 wt.% catalysts and methanol to oil ratio of 6:1 resulted product of 94% (Mansir et al., 2018). Production of biodiesel of microalgal oil with recombinant *Rhizomucor miehei* lipase expressed in Pichia pastoris, the conversion rates obtained with methanol oil ratio 3:1 to 5:1 (86.6%) and ethanol: oil ratio 5:1 (87.5%).

The presence of oleic acid in WFO, susceptible to oxidation due to its low activation energy (Wang et al., 2021), is predominantly responsible for the low yield observed in Figure 5. The oxidation reactions reduce the conversion of fatty acids to methyl esters, which are required for biodiesel production. In addition, the presence of water molecules bonded to WFO contributes decreased in biodiesel yield. Initially, a rise in the ratio of water to biomass yields an increase in biodiesel yield. However, once the yield reaches its optimum level, a higher water weight ratio reduces yield. This is because a high-water content negatively impacts an enzymes activity and stability (Li et al., 2013). In addition to the aforementioned factors, enzyme aggregation can also be caused by an enzyme concentration that is too high. This aggregation hinders enzymes' flexibility and decreases enzymes' binding efficacy to substrates, thereby decreasing the overall degradation efficiency (Fam et al., 2009). However, it is essential to note that enzyme concentration is not the only factor influencing biodiesel yield. The presence of the byproduct glycerol is an additional significant factor. Using the enzyme catalyst Pichia pastoris, glycerol synthesis is significantly lower than when using acid or base catalysts. The reduction in byproduct formation using the enzyme catalyst *Pichia pastoris* suggests that a low water content promotes the accumulation of glycerol as a byproduct (Salem et al., 2018).

However, at a lower enzyme concentration (1%), insufficient substrate molecules might occupy all available active sites. This underutilization of active sites can slow the reaction rate and reduce the yield. Increasing the enzyme concentration to 2% provides more available active sites for substrate binding, enhancing the efficiency of the conversion process and increasing the yield. However, the active sites may become saturated if the enzyme concentration is further increased to 3%. The surplus enzyme molecules can compete with the substrate for binding, leading to steric hindrance or altered enzyme-substrate interactions. This saturation effect diminishes the catalytic efficiency, resulting in a lower yield and diminishing returns.

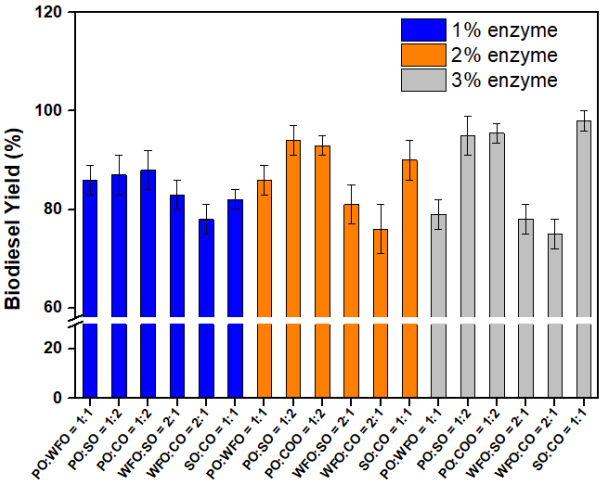

Fig. 5: Yield of the various composition of multi feedstocks with addition of, 1, 2, and 3 % enzyme

CONCLUSIONS

By combining various basic materials, the characteristics of biodiesel are altered. Using multiple feedstocks significantly impacts the density of the resultant biodiesel, which decreases. For the mixture of WFO and CO, the minimum density obtained is 861,1 kg/m^3. Multiple feedstocks result in higher viscosity values compared to ASTM standards when it comes to viscosity. The mixture of WFO and SO has the maximum recorded viscosity at 18,03 mm^2/s. The deposit number of biodiesels is also affected significantly by the blending of basic materials. With a value of 161.29, the mixture of PO and SO has the lowest number of deposited particles. In addition, the blending of raw materials affects on the iodine number of biodiesels, with the number decreasing in the case of multi-feedstock biodiesel. Nevertheless, it is important to note that the acidity of the multi-feedstock biodiesel exceeds the ASTM standards. Consequently, it is essential to contemplate merging this biodiesel with other substances to satisfy the required viscosity standards. In addition, the concentration of enzymes utilized in biodiesel production from multiple feedstocks affects the biodiesel's quality. According to the biodiesel quality analysis, the yield of multi-feedstock biodiesel increases with enzyme concentrations between 1% and 3%. Within the enzyme concentration range, biodiesel density tends to decrease, whereas its viscosity increases. In addition, adding enzymes at concentrations ranging from 1 to 3 % increases the Cetane number of biodiesels.

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