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Promising Abilities of Fungal Lipases of Aspergilli Strains in the Production of Biodiesel from Plant Oil Wastes

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Abstract: Currently, there exists a significant need for alternate sources of petroleum fuel due to escalating environmental challenges and diminishing global oil reserves. Biodiesel, an environmentally friendly and sustainable fuel, is one of the most adaptable alternatives to petroleum fuel. This study's goals were to get biodiesel from three oils (olive, palm, and jojoba) and see how well lipases work as biocatalysts for the esterification process. The production of biodiesel was obtained from two fungal strains, *Aspergillus niger* MH079049.1 and *A. niger* MH078571.1, and four distinct fatty acids and four organic solvents were used. The study determined the optimal reaction conditions to be a reaction time of 24 h, an enzyme concentration of 3 mL, and an oil-to-methanol ratio of 3:1. This study showed that lipase works well in making biodiesel, with an impressive esterification yield of 99%, and the highest level of productivity was attained when olive oil and jojoba oils were subjected to an incubation time of 18 h and 22 h, respectively, in the presence of palm oil.

Keywords: fatty acid methyl esters (FAME); lipase; transesterification; olive oil; jojoba oil; palm oil; *Aspergillus niger*

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1. Introduction

Enzymes aid in numerous life-sustaining biological processes by decreasing activation energy without inducing permanent alterations. Enzymes are present in microorganisms, plants, and animals. The growing demand for industrial enzymatic production has caused a shift in focus from plant and animal sources to microbial sources [1]. Microbial enzyme production is faster, cheaper, scalable, and genetically manipulable [2]. Over 50% of industrial enzymes come from fungi [3]. In filamentous fungi, fungal enzymes have a huge synthesis capacity, simpler purification and separation methods, and excellent catalytic activity with good stability in harsh environments [3]. Lipase from fungi is crucial for commercial use. Many filamentous fungi may manufacture lipase to break down fats and oils in polluted soil, waste oils, dairy products, and seeds. To be conveniently retrieved from fermentation, fungal enzymes are released outside the cell [4,5]. The filamentous fungus may create extracellular lipase in the fermentation broth, solid, and submerged fermentation, and employ a broad variety of agricultural and other waste products as nutrients [6].

Studies have found that numerous Aspergillus sp. generate lipase, which has many industrially important features including pH, thermal stability, and high specificity, and different purification methods have been applied [4].

In the presence of a catalyst, transesterification of fatty acids with short-chain alcohols, such as methanol or ethanol, yields a long chain of fatty acids such as methyl ester, ethyl

ester, or single alkyl ester. It is possible to produce biodiesel and glycerin from plant lipids. The majority of plant oils contain palmitate, stearate, oleate, linoleate, and linolenate as the main essential fatty acids [7–9]. Monounsaturated fatty acids include oleate, whereas polyunsaturated fatty acids include linoleate and linolenate. Most plant oils utilized commercially as biodiesel feedstocks include a large amount of polyunsaturated fatty acids, which decreases stability, cetane number, and biofuel use [10]. Biodiesel with high monounsaturated fatty acid (oleate) content has superior ignition quality, NOx emissions, and oxidative stability [11]. Thus, high-oleic acid and low-palmitic acid plant oils make good biodiesel [12]. In addition to plant oil, biodiesel is made from inedible animal fats, leftover cooking oils, and vegetable and animal oil refinery waste. These feedstocks drastically lower biodiesel production costs. Preventing food–energy conflict. It is one of the most sustainable and viable alternatives to fossil fuels and a vital step in decreasing pollution and reusing waste oils [13].

The European Union, the world's biggest biodiesel producer, produced approximately 17,000 million liters in 2010 from 950 L in 2000. It produces 53% of worldwide biodiesel. By 2011, biodiesel made up over 20% of global biofuel output, up from 5% in 2000. Biodiesel output is expected to reach 41,000 million liters in 2022, according to the UN. The higher production rate of biodiesel might be achieved using lipase known for its thermal stability and capacity to survive in the presence of short chain alcohols [14,15].

Biodiesel, which is neither flammable nor explosive and produces few gasses when burnt, is produced worldwide using alkaline catalysis transesterification. It is made via reciprocating esterification of vegetable or animal fat. After filtering, it is heated to 70 °C for an hour to combine alcohol with oil or fat using an alkaline catalyst, and with NaOH or KOH to adjust pH. The mixture is left until biodiesel floats to the top and glycerol settles at the bottom. This chemical procedure has drawbacks. The bottom needs filtration to neutralize the catalyst and separate biodiesel and glycerol. The reaction produces a lot of soap, complicating this final step. This method generates a lot of effluent that must be treated. Due to their sensitivity to raw material purity, alkaline catalysts need raw materials devoid of phosphatides, free fatty acids, and water, making them costly and ecologically damaging [16].

Lipase transesterification is becoming increasingly desirable because it offers benefits over alkaline catalysis transesterification, including the production of high-quality biodiesel that disables SoapAction due to a high-performance purification process and low temperature range 30–40 °C which eliminates the requirement for 246 °C temperatures and expenses. [17,18].

It promotes triglyceride fatty acid cross-esterification and FFA esterification with alcohol. Oils or fats with high FFA levels may be utilized to manufacture biodiesel. Glycerol recovery and biodiesel purification are straightforward since lipases create fatty acid esters without soap. It is selective, needs moderate reaction conditions, and generates minimal side products and wastewater [16].

The lipase-catalyzed catalytic exchange process is becoming more attractive because this process has several advantages over chemical catalysis. With the addition of microbial lipase, we do not need temperatures up to 246 °C and the associated costs.

The most extensively utilized enzymes for biodiesel synthesis are those from *P. fluorescens* (Lipase AK, Amano), *B. cepacia* (Lipase PS, Amano), and *T. lanuginosus* (Lipase LA201 and Lipopan 50BG, Novozymes). (Lipozyme TL IM, Novozymes) *T. lanuginosus* and *R. miehei* fixed lipase [19].

Transesterification through alkaline catalysis dominates the worldwide biodiesel industry, with limited Lipase transesterification usage. China was the first to run a lipase-based biodiesel production in 2006. Research conducted by Du and Liu [20] in 2012 has shown that lipase enzymes can make biodiesel. Brazil, a biodiesel pioneer, established a large-scale pilot plant to demonstrate this feat.

Biodiesel is made from lipase-producing fungi and yeasts such *Aspergillus niger*, *Candida antarctica*, *Candida rugosa*, *Rhizomucor miehei*, *Rhizopus oryzae*, and *Thermomyces lanugi-*

nosus [21]. A stable lipase from *C. antarctica* is used in the substitutional esterification of methanol and soybean oil to make biodiesel [22]. Winayanu et al. [23] found that *C. rugosa* lipase (CRL) was stable, making it a good biocatalyst for enzyme-catalyzed biodiesel production. Su et al. [24] studied CRL lipase catalysis in ionic liquid for biodiesel production from Chinese tallow kernel oil.

Thailand, Malaysia, and Indonesia are rich in palm oil. Palm oil mills produce several byproducts and waste materials that, if not properly handled, may harm the environment. Each ton of palm oil processed produces 0.65 tons of palm oil mill effluent, according to Jong et al. [25]. Reports reveal that palm oil mill effluent (POME) contains carbohydrates, protein, and mineral salts that may support yeast growth. A variety of Yarrowia lipolytica strains were screened for lipid and lipase production. Palm oil mill effluent (POME) was used as an efficient nutritional medium for this screening. We chose the best strain and improved the nutritional medium. Since yeast cells and lipase are active and stable across pH and temperature, they were collected after culture under optimal conditions. Mustar [26] found that yeast lipids and lipases may be directly transesterified to make biodiesel without cell drying or lipid extraction.

The lipase enzymes of *Aspergillus niger* MH079049.1 and MH078571.1 were tested for esterification in the presence of various fatty acids and organic solvents and for biodiesel production utilizing olive, palm, and jojoba oils.

2. Materials and Methods

2.1. The Source of Fungal Strains Isolates

The two strains that exhibited the maximum lipase production were acquired from a prior investigation conducted by Alabdalall et al. [27]. The identification of the two fungal isolates was conducted using partial 18S rDNA sequencing at the Institute for Research and Medical Consultations (IRMC), located at Imam Abdulrahman Bin Faisal University in Dammam, Saudi Arabia. The isolates were determined to be *Aspergillus niger*, with GenBank Accession Nos. MH078571.1 and MH079049.1. The fungi were cultivated as pure cultures on Potato Dextrose agar slants and afterward kept at a temperature of 4 °C.

2.2. Lipase Production by Submerged Fermentation

In Tween 80 broth, *A. niger* MH078571.1 and MH079049.1 produced lipase [28], when inoculating a sterile medium with 2 mL of 3×10^7 spores/mL fungal conidial suspension in the mineral salt solution. Both cultures were incubated at 25 ± 2 °C in a rotary shaker at 200 rpm for 5 days. After incubation, Whatman's filter paper no. 1 filtered fungal growth. Spectro UV-VIS Double spectrophotometers were used to measure lipase synthesis in clear supernatants from filtered cultures centrifuged at 1200 rpm for 30 min at 4 °C.

2.3. Lipase Production Assay

Lipase production is measured using pNPP hydrolysis. In this study, 100 µL of the two clear supernatants were incubated for 15 min at 30 °C with 800 µL of 0.25% polyvinyl alcohol (PVA) solution at pH 6.5 and 100 µL of 3 mM pNPP in isopropanol. Following this, 500 µL 3 M HCl stopped the process and 500 µL of each supernatant were added to 1 mL of 2 M NaOH after incubation. Spectro UV-VIS Double measured absorbance at 410 nm, and 1 µM of pNPP released per minute determined the enzymatic activity unit (U). Oliveira et al. [29] used the standard curve enzyme activity measure.

2.4. Enzyme Characterization

Following [30]'s procedure to determine the temperature effect on the enzyme activity and its stability, the supernatant mixtures were incubated at different temperatures ranging from 25 °C to 70 °C and at 1, 2, 4, and 24 h. According to our previous study [27], the optimum temperature was determined as 45 °C and 55 °C with a pH 8 for the *A. niger* MH078571.1.1 and *A. niger* MH079049.1.1, respectively. The supernatant mixtures were also incubated at pH ranges 3, 4, 5, 6, 7, 8, 9, and 10 [31], with 100% and 50% concentrations

of organic solvents 1:1 ratio (*v*/*v*) for enzyme and organic solvent (methanol, ethanol, isopropanol, butanol, acetone) [32], various concentrations of surfactant solutions (tween 80, tween 20, sodium dodecyl sulfate (SDS)) [33], and metal ions ($CaCl_2$, NaCl, KCl, K_4, NH_4Cl, Mg SO_4, Cu SO_4, $ZnSO_4$, EDTA) [31]. These factors have been studied to identify the optimum conditions for the lipase activity and its stability to be used in various industrial and commercial applications [34].

2.5. Lipase-Catalyzed Esterification and Transesterification Reactions

Esterification reactions catalyzed via lipase were performed using different organic acids and alcohols. Lipase was obtained from a 7-day SSF conducted under optimum conditions, with the fermented substrate containing the lipase simply being lyophilized. Reactions occurred in 15 mL falcon tubes with a 5 mL mixture of organic acid and alcohol in a 1:1 molar ratio in a total volume of 5 mL, and 20% (*w*/*v*) of biocatalyst was added to the mixture. The Falcon tubes were incubated in a shaker at 200 rpm, 37 °C for 24 h, and 5 mL of 1:1 (*v*/*v*) acetone with absolute ethanol was added. Conversion (%) of the organic acid to ester was determined via titration with 0.1 M NaOH Sodium hydroxide (NaOH). The highest conversion rate of esterification (100%) was obtained with a combination of decanoic acid with butanol. The minimum lipase concentration needed to achieve 100% conversion in 24 h was determined. For that, 20% (*w*/*v*), 10%, 5%, and 1% of biocatalyst were used, and the time course profile of conversion over reaction time was obtained. And the effect of adding a buffer to the biocatalyst in the esterification reaction was studied by adding a low amount of lyophilized biocatalyst, 5% (*w*/*v*), to Eppendorf tubes, then resuspending it in 1 mL of Britton Robinson universal buffer (0.04 M H_3BO_3, 0.04 M H_3PO_4, 0.04 M CH_3COOH, and 0.2 M NaOH) at pH values of 5–9 before it was lyophilized again. Finally, its performance was analyzed by observing the reaction of decanoic acid with butanol [35].

For this research, gas tubes with a volume of 10 mL containing 0.55 g of various oils, such as olive oil, jojoba oil, and palm oil were used for transesterification reaction investigation. The oil-to-methanol molar ratio was 3:1 and lipase (0.3% based on oil weight) was added to the reaction mixture and the reaction time began. The mixture was incubated for 6 to 26 h at room temperature in a shaking incubator for 24 h, then in a rocking incubator at a temperature of 35 °C with a stirring rate of 180 rpm for both incubators. Samples were taken at different time intervals and 100 µL was removed and mixed with 100 µL of hexane, and subjected to agitation in a tube vibrator, followed by centrifugation. The upper layer was then extracted and analyzed using Gas Chromatography-mass spectrometer QP2010 SE (Shimadzu Made in USA). Sample preparations for chromatography were performed according to a previously reported and modified method [33,35–37]. Methyl esters of palmitic, stearic, oleic, and linoleic acids were used as standards and purchased from Sigma.

3. Results

3.1. Utilizing Lipase Enzyme as a Catalyst in an Esterification Reaction

Table 1 illustrates the enzymatic capacity to facilitate ester formation via the bonding of the ester with the solvent. The esterification of fatty acids was observed to occur at a rate of 99%, with the highest conversion rate observed for oleic acid when using methanol as the solvent. In contrast, the formation of esters using butyric acid and benzene alcohol was found to be the least efficient among the different fatty acids tested in the presence of lipases. Specifically, *A. niger* MH079049 achieved a conversion rate of 98.99%, while *A. niger* MH078571 achieved a slightly higher conversion rate of 99.01% (Figure 1).

Table 1. Lipase enzyme formation capacity between the two fungal strains.

Organic Acid	Organic Solvent	Isolates			
		A. niger MH078571		*A. niger* MH079049	
		FFA %	Ester Percentage %	FFA %	Ester Percentage %
Butyric acid	n-Butanol	0.755	99.25	0.812	99.19
	Iso-butanol	0.861	99.14	0.919	99.08
	Benzyl alcohol	0.986	99.01	1.016	98.99
	Methanol	0.845	99.16	0.871	99.13
Propionic acid	n-Butanol	0.666	99.33	0.707	99.3
	Iso-butanol	0.721	99.28	0.721	99.28
	Benzyl alcohol	0.664	99.34	0.699	99.30
	Methanol	0.673	99.33	0.704	99.3
Lactic acid	n-Butanol	0.624	99.38	0.944	99.06
	Iso-butanol	0.681	99.32	0.632	99.37
	Benzyl alcohol	0.774	99.23	0.678	99.32
	Methanol	0.798	99.20	0.816	99.18
Oleic acid	n-Butanol	0.214	99.79	0.165	99.83
	Iso-butanol	0.365	99.64	0.391	99.61
	Benzyl alcohol	0.252	99.75	0.248	99.75
	Methanol	0.192	99.80	0.143	99.86

FFA = Free fatty acid percentage %.

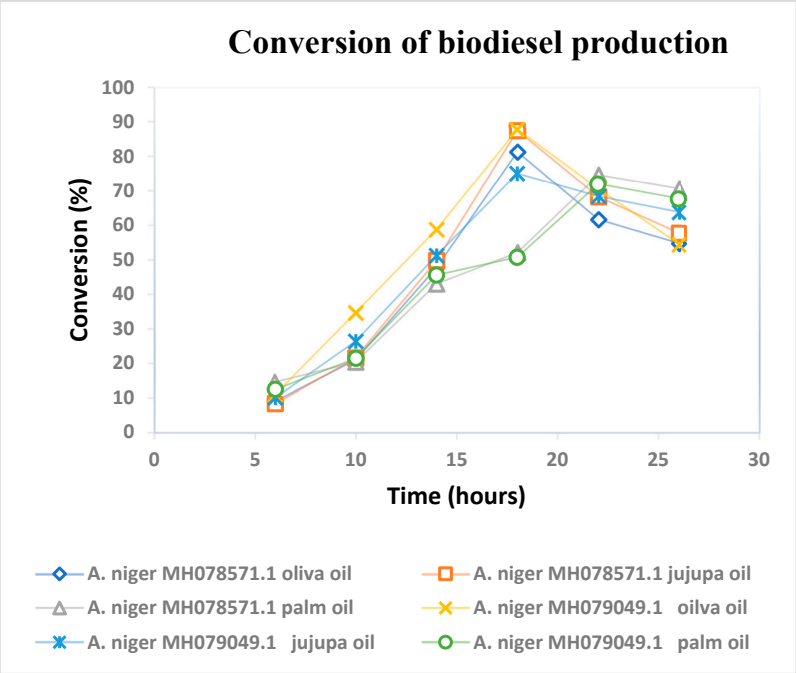


Figure 1. Methyl ester concentration during biodiesel production as a function of lipase catalysis. (*A. niger* MH078571.1 olive oil = blue color; palm oil = green color; jujupa oil = orange color and *A. niger* MH079049.1 jujupa oil = violet color; olive oil = blue color; palm oil = green color).

3.2. Lipase Enzyme on Catalyzing Biodiesel Production

The inclusion of fungal lipase has been shown to enhance the synthesis of biodiesel when olive, jojoba, and palm vegetable oils are used (refer to Table 2). The optimal production of olive oil and jojoba was observed at 18 h for both substances. Subsequently, the production declined as the formation of methyl gas increased. Specifically, the fungal strain *A. niger* MH078571 achieved a methyl gas formation of 81.18% and 87.44% for olive oil and jojoba, respectively. On the other hand, the other strain (*A. niger* MH079049) achieved a methyl gas formation of 87.77% and 74.96% for olive oil and jojoba, respectively. Our findings indicate that the optimal duration for methyl gas generation in the presence of palm oil is 22 h. Specifically, *A. niger* MH078571 achieved a production rate of 74.51%, while *A. niger* MH079049 reached 71.94%.

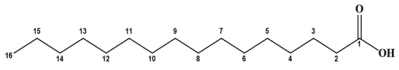
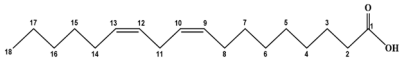
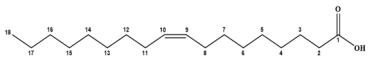
Table 2. Methyl ester concentration during lipase catalyzed biodiesel production at various times.

Time Conversion	*A. niger* MH078571			*A. niger* MH079049		
	Oliva Oil	Jojoba Oil	Palm Oil	Oilva Oil	Jojoba Oil	Palm Oil
6 h	8.95	8.18	14.54	10.80	9.89	12.36
10 h	20.97	21.52	20.37	34.61	26.35	21.36
14 h	47.68	49.73	42.98	58.70	51.16	45.53
18 h	81.18	87.44	52.01	87.77	74.96	50.71
22 h	61.58	68.18	74.51	70.50	68.34	71.94
26 h	54.65	57.62	70.56	54.34	63.72	67.66

3.3. Mass Spectrometry

The chemical structures of the parent acids (palmitic, linoleic, and oleic acid) were depicted using Chem-BioDraw Ultra, v.18.0. Further information, including the presumed chemical formulas with corresponding molecular weights and the anticipated mass fragmentation in mass spectrometry fractions, is provided in Table 3. Figures 2–4 show the mass spectra of methyl ester. The methyl esters' molecular ion (parent) peak and base peak (the tallest peak in the spectrum) are displayed in Table 3. The predicted molecular ion peaks for methyl palmitate, methyl linoleate, and methyl oleate were detected at m/z 272, 294, and 296, respectively. In mass spectrometry, the parent peak (base peak) for methyl palmitate, is observed at m/z = (CH_3-CH_2-CH_2-CH_2-, ~57). (100%, $C_4H_9^+$) (Figure 2), whereas the mass spectrum of methyl linoleate and methyl oleate were observed at m/z = ($CH_3CH_2CH_2CH_2$, 57) (100%, C_4H_9-) and at m/z = (CH_2=$CHCH_2CH_2$, 55) (100%,C_4H_7-), respectively, and depicted in Figures 3 and 4, respectively.

Table 3. Chemical composition, Molecular ion, and base peaks of fatty acid methyl ester (FAME).

Name of Fatty Acid Ester	Molecular Formula	Chemical Structures of the Parent Acids	Molecular Ion Peak (*m*/*z*) (Found)	Molecular Weight (Calc.)	Base Peak (*m*/*z*)
Methyl palmitate	$C_{16}H_{34}O_2$		272	270	57
Methyl linoleate	$C_{18}H_{34}O_2$		294	298	57
Methyl oleate	$C_{18}H_{36}O_2$		298	297	55

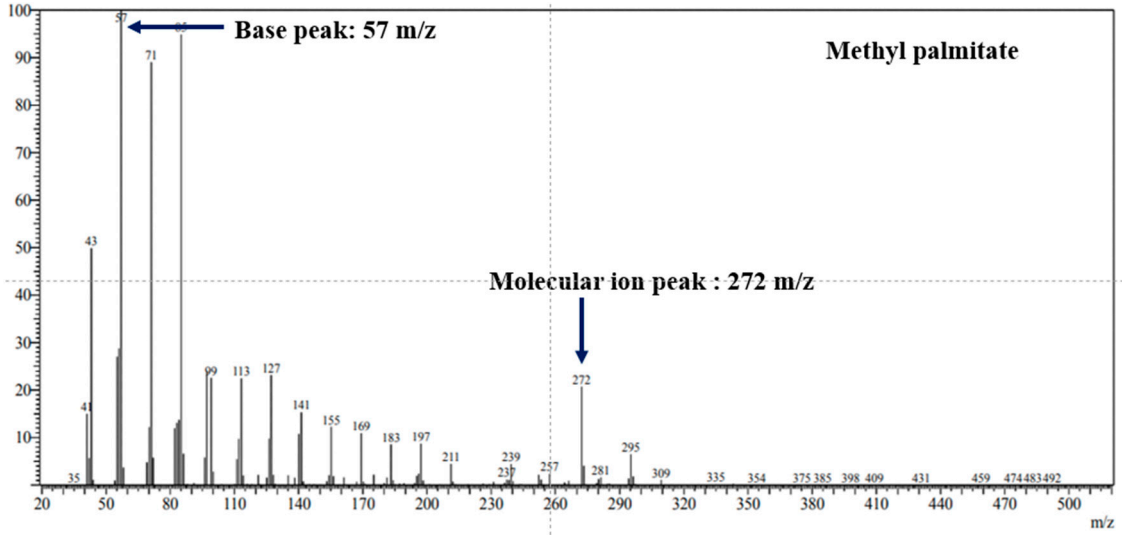


Figure 2. Mass spectrum of methyl palmitate.

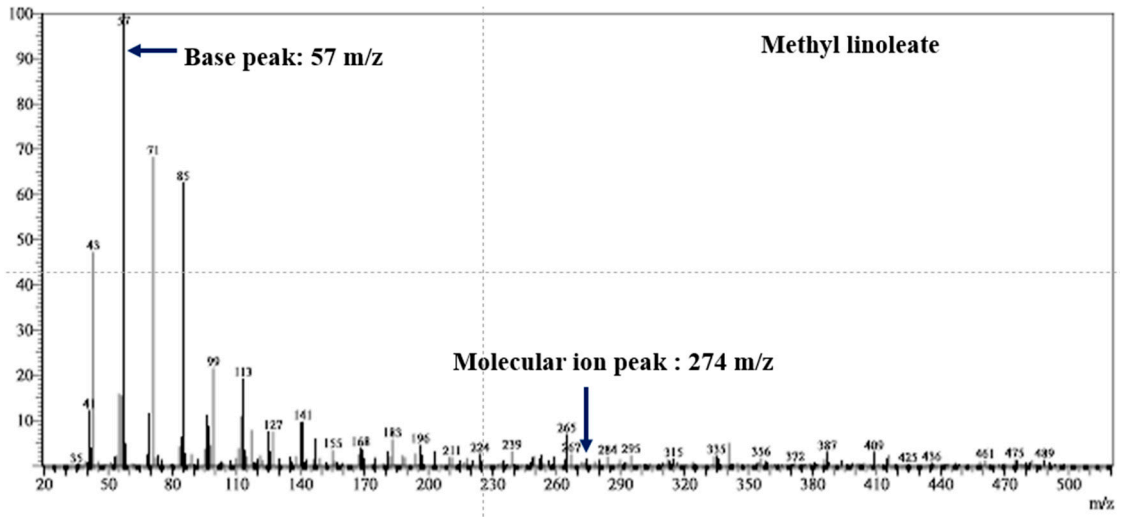


Figure 3. Mass spectrum of methyl linoleate.

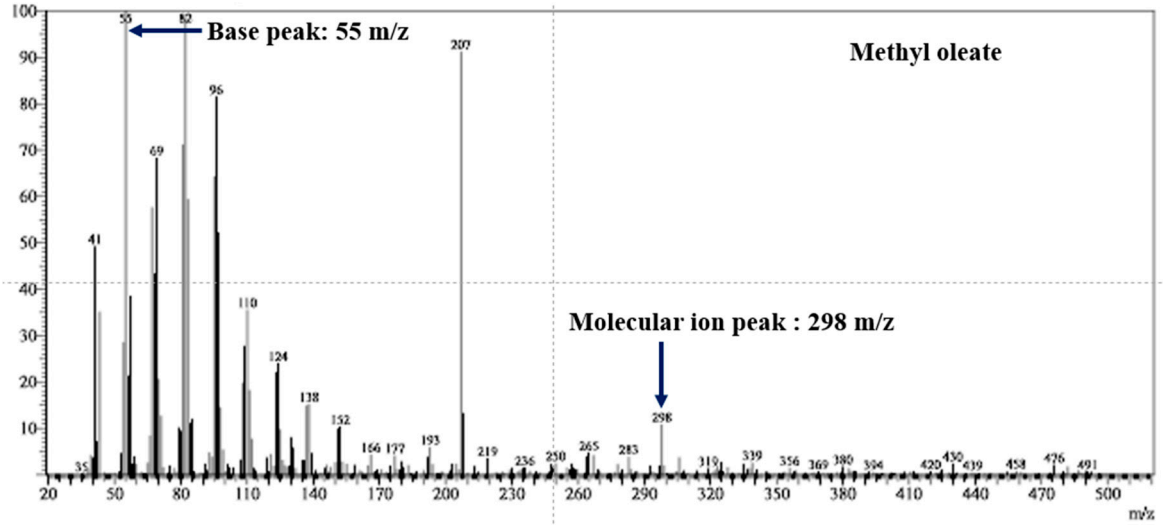


Figure 4. Mass spectrum of methyl oleate.

4. Discussion

According to Haki and Rakshit [17], bacteria and fungi that are found in many habitats and have strong resilience to endure challenging circumstances provide valuable resources for use in demanding industrial operations. The food processing industry is facilitated by the use of lipase enzymes, which play a crucial role in modifying the positioning of fatty acid chains within glycerides. This enzymatic process involves the substitution of existing fatty acids with new ones, which is to say the conversion of less-desired fats into more economically viable fats [38,39].

Esterification reactions are often conducted in the presence of non-absorbable organic solvents and under circumstances of low water content. This choice of reaction medium is favored due to its ability to enhance substrate solubility [40].

The lipase enzyme's capacity to facilitate the esterification process was confirmed by conducting experiments using four distinct fatty acids and four different solvents. The stability exhibited by the crude enzyme when prepared in water-insoluble solvents is associated with its capacity to facilitate the synthesis of esters. The enzyme demonstrated significant conversion rates, exceeding 99% within a 24-h period, indicating its potential applicability in the production of esters utilized for the creation of food flavors. This observation aligns with the findings of a study conducted by [41]. The ester compounds

butyl butyrate, butyl acetate, and butyl oleate were synthesized using lipase as a catalyst, resulting in yields of 70%, 14%, and 40%, respectively. The results of this study demonstrated the synthesis of ester compounds via the activation of the lipase enzyme. And the observed percentage of ester production was shown to be dependent on both the solvent used and the specific fatty acid utilized, which is in agreement with results of [35]'s study.

Vegetable oils derived from non-edible plants exhibit robust development in dry and semi-arid climates, necessitating nutrient-rich soils with minimal moisture levels for optimal cultivation. According to [42], the researchers reached the conclusion that non-edible raw materials should be used in the manufacturing of biodiesel as a viable alternative fuel with applications across several industries. Jojoba oil, being a non-consumable oil derived from a desert plant, has a greater biodiesel output when subjected to lipase enzymes. The economic significance of the issue being investigated is considerable, since several lipase enzymes need a time range of 5–72 h to achieve optimal biodiesel production output [43]. The highest lipase production of both strains was obtained after 18 h for olive oil and jojoba and 22 h for palm oil. The production rate was 81.18%, 87.44%, and 74.51% for *A. niger* MH078571.1, and 87.77%, 74.96%, and 71.94% for *A. niger* MH079049.1 for olive, jojoba oil, and palm oil, respectively. Therefore, the lipase enzyme produced by the two strains is considered an effective enzyme that needs a short time to perform the transesterification process, compared to the optimal time to obtain the highest production of biodiesel in other studies while catalyzed using lipase with soybean oil and olive oil with methanol to produce biodiesel. The maximum production was reached after 24 and 36 h for soybean oil and olive oil, respectively [44]. Sahoo et al. [44] mentioned that among the various oils used for biofuel production, the maximum yield of 44.2% and 76.8% were observed with sesame after incubation for 6 and 12 h, respectively. Likewise, 12 h after incubation, production reached 73%, 61%, and 59% with palm oil, sunflower oil, and peanut oil, respectively. Kuo et al. [45], also found that the rate of biodiesel catalysis by CRL2 in the presence of methanol throughout 8, 16, and 24 h was 94.5%, 94.8%, and 95.3%, respectively, with 24 h being considered the period.

The results of [33] indicated that biodiesel was produced at its highest value in the presence of olive oil after 18 h of up to 78% with a 62% catalyzed from lipase from *B. licheniformis* KM12, whereas at the current study, a higher level of productivity was attained after 18 h up to 81.18% with the fungal strain *A. niger* MH078571 and up to 87.77% with *A. niger* MH079049.

Methyl-Palmitate, linoleate, and oleate represent distinct fatty acid esters that formed from the esterification reaction occur between fatty acid and alcohol as shown in the following reaction (1). These fatty acid derivatives are produced from parent acids (palmitic, linoleic, and oleic acid) reacting with methanol via biological esterification (2). And because each of these fatty acid derivatives is characterized by unique features discernible in its mass spectrometry profiles, they may be used as an analytical reference standard for the quantification of the analyte in biodiesel samples using chromatography techniques [46]. However, the differences in structure and composition of these parent acids lead to expected variations in the mass spectra depicted in Figures 3 and 4.

$$\text{R-COOH} + \text{R}'\text{-OH} \rightleftarrows \text{R-COOR}' + \text{H}_2\text{O} \quad (1)$$

$$\underset{\text{Palmitic acid}}{\text{CH}_3(\text{CH}_2)_{12}\text{COOH}} + \underset{\text{Methanol}}{\text{CH}_3\text{OH}} \rightarrow \underset{\text{Methyl palmitate}}{\text{CH}_3(\text{CH}_2)_{12}\text{COOCH}_3} + \underset{\text{water}}{\text{H}_2\text{O}} \quad (2)$$

It is worth noting that the study found high molecular weight (270 to 300 m/z) of the mass spectra of methyl ester detected in biodiesel in the absence of a SoapAction, and this is in agreement with what was reported by [47]: that a greater portion of fatty acid of high molecular weight is present when a saponification value is low. A significant saturated methyl ester has been detected in biodiesel (methyl palmitate), which shows significant fragments and appears at m/z = 57 as the base peak (100%), which results from the breakage of the C12–C13 bond to give [$CH_3CH_2CH_2CH_2$]. It is interesting to note

that methyl palmitate showed a fragmentation pattern identical to methyl linoleate with two double bonds. And this is in agreement with [48]. The mass spectrum of methyl oleate shows a most (abundant/parent) peak which corresponds to C_5H_{11}, while the highly intensive fragmentation peak at 207 (91.5%), assigned for at the $C_{13}H_{23}O_2$ ~ m/z: 207 (91%, $C_{13}H_{23}O_2$), arises from the breakage of C16–C17 with a loss of ethane due to the loss of pentyl (C_5H_{11}-) and along with further loses a carbon dioxide (CO_2) and methane (CH_4) molecules.

Molecular weight of palmitic methyl ester spectrum was obtained from 270 to 275 m/z, while linoleic & oleic methyl ester spectra were observed from 294 to 300 m/z. Linoleic & oleic methyl ester spectra were observed from 294 to 300 m/z.

5. Conclusions and Future Prospects

This study esterified biodiesel from three plant oil waste (olive, palm, and jojoba) using lipases. *Aspergillus niger* MH079049.1 and MH078571.1 created biodiesel from four fatty acids and four organic solvents. 24 h, 3 mL enzyme concentration, and a 3:1 oil-to-methanol ratio were ideal reaction conditions, according to studies. And GC-MS analysis showed that methyl palmitate, methyl linoleate, and methyl oleate were found at 272, 294 and 296. Olive and jojoba oils incubated for 18 and 22 h in palm oil generated the highest level of productivity. Biodiesel synthesis using lipase transesterification has some benefits over alkaline catalysis transesterification including the production of high-quality biodiesel that disables SoapAction under high performance and under purification process. But some of the main disadvantages that hinder lipase transesterification development in biodiesel industry are high operating expense and poor enzyme stability. In this study, lipase enzyme has been successfully introduced and its stability and activity have been improved to perform the transesterification process in a short time, yielding 99% esterification. Whereas the high operating expense can be partly solved by using it to produce B20 biodiesel blend due to the advantages it offered, in addition to the urgent need to develop its process by future research to reduce operating costs while improving operational efficiency such as immobilization of lipase research using several supporting materials such as silica nanoflowers, pickering emulsion, the artificial neural network, and semi-solid-state fermentation.

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