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Research article

Assessment of myco-diesel and a comparison of their quality indicators with global standards

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ABSTRACT

The quality standards of good biodiesel are directly proportional to its compatibility with internal combustion engines without the need for additional modifications. This study aimed to investigate the acceptability of biodiesel derived from fungi based on biodiesel quality characteristics according to international standards. Twelve published fungal isolates, identified as oleaginous fungi, were targeted, and their suitability for use in internal combustion engines was predicted and compared to these standards. The results showed that, despite relative variation in fatty acid methyl esters among the fungal isolates, the biodiesel derived from fungi achieved a cetane number ranging from 53 to 68 for most isolates. The kinematic viscosity showed acceptable results for 10 isolates according to American Society for Testing & Materials (ASTM)/European Norm (EN) International Organization for Standardization (ISO) standards, while the ANER/AFUS isolates showed unacceptable values according to these standards. The density value for most fungal isolates showed compliance with ASTM/EN ISO standards. The diesel fuel derived from fungi exhibited very acceptable values for cloud point and Cold filter plugging point. Due to the high concentration of unsaturated fatty acids, the diesel fuel derived from fungi may be susceptible to oxidation, which could affect its oxidative stability and shelf life. Furthermore, the diesel fuel derived from fungi showed a higher heating value exceeding 20 in most isolated samples. The saponification values for most isolated samples ranged between 100 and 200 mg KOH/g of lipids. Based on the European standard, the iodine value of the diesel fuel derived from fungi did not exceed 120 g of iodine I_2/100 g.

1. Introduction

Growing global energy demand requires sustainable solutions, the most important of which is diversifying energy sources in addition to reducing dependence on fossil fuels. Furthermore, excessive consumption of petroleum fuels can lead to severe environmental/health consequences [1]. Increased fossil fuel consumption results in significant external costs, including increased air pollution and associated health impacts. In many countries, the elimination of these subsidies has led to significant reductions in air pollutants, such as nitrogen oxides and particulate matter. Addressing these structural issues is critical [2].

The idea of producing biodiesel practically originated with Rudolf Diesel, who first used peanut oil as a vegetable oil. With the significant increase in pollution in nature due to incomplete combustion and fossil fuel residues, the idea of biodiesel production has resurfaced. In recent years, many researchers have explored using animal fats, vegetable oils, and lipids from microorganisms such as algae as a promising alternative for producing clean, renewable energy [3].

Biodiesel has emerged as a potential alternative to biofuels in the face of the challenge of fossil fuel depletion [4]. Biodiesel can be produced from a variety of sources, including waste materials, used cooking oil, vegetable oils, animal fats, algae, and other organic waste [5].

With an effective focus on the recycling axis, biofuel extracted from biomass can be considered a promising fuel that can be produced in its solid, gaseous or liquid form. As a result, what distinguishes this fuel is that it is free of pollutants, biodegradable and renewable [6,7].

The developments in biodiesel can be described as 4 generations. The first generation includes sources based on vegetable oils such as palm oil, rapeseed oil, and others. The second generation includes waste oils, whether agricultural or industrial waste. On the other hand, the third generation relied on oils derived from algae and microorganisms. The fourth generation sought to employ aspects of genetic modification in achieving optimal biodiesel production based on microorganisms or others [8].

Due to some limitations of using biodiesel from animal/plant lipids, many researchers have turned their attention to oleaginous microorganisms as a reliable alternative resource. Oleaginous fungi are of great

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interest to researchers due to their ability to accumulate large amounts of lipids (more than 20% of the dry weight of the biomass) as well as the properties of the polyunsaturated fatty acids they contain [9].

Studying the properties of biodiesel based on the content of fatty acid methyl esters (FAME) can help in selecting the most suitable oils for biodiesel production. For example, the cold flow properties of biodiesel, including the cold filter plugging point (CFPP), cloud point (CP) [10], cetane numbers (CN) and viscosity (KP), are critical to determining the suitability of biofuels for internal combustion engines [11]. The esters content in fuel indicates the amount of FAMEs present, therefore, a very important indicator of biodiesel quality. Low esters indicate that unreacted compounds, such as triglycerides, or process-related compounds, such as catalysts, remain in the fuel. Low levels may also indicate contamination with non-FAME compounds. These impurities can cause fuel filter clogging, engine deposits, or other problems[12]. Among the most important fatty acids that are important for producing highly efficient biodiesel are: hexadecanoic acid (C16:0), monounsaturated omega-9 fatty acid such as oleic acid (C18:1), and polyunsaturated, omega-6 fatty acid such as linoleic acid (C18:2)[13].

Ultimately, ensuring that biodiesel properties comply with international standards, such as American Society for Testing & Materials (ASTM) or European Norm (EN), is the biggest challenge in producing high-quality biodiesel that can be used in internal combustion engines without modification [14]. Considering the studies related to the possibility of producing biodiesel from fungi, the published studies lack advanced investigation related to studying the properties of biodiesel from fungi according to global standards. We selected 12 fungal isolates that were described as oleaginous isolates. This study aims to investigate the properties of biodiesel produced from these isolates, based on the quality characteristics of biodiesel based on ASTM/EN standards, comparing the most efficient species/strains with others, predicting their suitability for use in internal combustion engines, as well as their advantages and disadvantages according to the approved standards.

2. Materials and methods

2.1. Oleaginous fungal isolates selection

Based on their lipid profiles, oleaginous fungal samples from previously published research papers were targeted. *Aspergillus fumigatus* strain *AB* (code: AFAB), *Aspergillus terreus* strain *KAIN1* (code: ATKA), *Cladosporium ramotenellum* strain *ZST3* (code: CRZS), and *Lichtheimia corymbifera* strain *K16* (code: LCK16) were selected based on[15]. Whereas the oleaginous fungi *Aspergillus niger* (code: ANIG), *Aspergillus flavus* (code: AFLA), *Aspergillus ochraceus* (code: AOCH), and *Penicillium chrysogenum* (code: PCHR), were selected based on[16]. On the other hand, *Aspergillus terreus* (code: ATER), as an oleaginous fungi were selected according to[17]. While *Aspergillus niger* (code: ANER), *Aspergillus flavus* (code: AFUS) and *Aspergillus terreus* (code: ATRE) were selected based on[18].

2.2. Myco-diesel properties

Based on FAMEs profiles of published oleaginous fungal studies included in 2.1, the properties of biodiesel produced from oleaginous fungi were investigated. This was done by considering the characteristics associated with biodiesel quality, the scientific efforts related to calculating these characteristics, and the parameters, references, and properties shown in Table 1.

2.3. Acceptability of myco-diesel

A comparative study was conducted on the properties of biodiesel produced from oleaginous fungi against global-local criteria to determine its suitability, efficiency for use in internal combustion engines (compression Ignition engines) and determine its practical acceptability. The most efficient isolate among the oleaginous fungi studied was identified. Comparison criteria were in light of the ASTM (code: ASTM) [25], EN standards with an International Organization for Standardization (ISO) (code: EN ISO) [12], EN 14214 [10] and Marketing Specifications Guide of Iraqi Petroleum Products[26] (code: Nat In) for diesel fuel.

2.4. Statistical analysis

The statistical analysis was conducted using SPSS ver. 25 [27] at a significance level of 0.05 to statistically present the research results.

3. Results and discussion

3.1. FAMEs composition of oleaginous fungi

Statistically, the results of the normal distribution analysis of the FAMEs data showed that they were normally distributed (Fig. 1). The results of the post-statistical analysis of the FAMEs of the oleaginous fungi that were studied showed that there were large significant differences between both 6 groups of identified FAMEs and fungal isolates, with a significant value of less than 0.001. Although the proportions of the identified FAMEs varied, most fungal isolates demonstrated their ability to accumulate FAMEs represented by Tetradecanoic acid (14:0), Hexadecanoic acid (16:0), Palmitoleic acid (16:1), Stearic acid (18:0), Oleic acid (18:1) and Linoleic acid (18:2). Differentially, the fatty acids (16:0, 18:1, 18:2) were most frequent compared to other groups of FAMEs (Fig. 2).

Built upon fungal isolates, the isolates ANIG, ATKA, LCK16 and ATRE had the highest FAMEs accumulation among the twelve isolates studied, while the isolate ANER was the least frequent. Many researchers have sought to produce biodiesel as a promising alternative to fossil fuels from micro-organisms, fungi on several levels. Organisms that have a high capacity to accumulate FAMEs, such as (C14:0, C16:0, C16:1, C18:0, C18:1) have received increasing attention from researchers [28], the results of this study are consistent with the current

Table 1
Properties targeted for myco-diesel study

No.	Properties	Abbreviation	Unit	Ref.
1	Cetane number	CN	Relative number	[19]
2	Kinematic viscosit	KV	mm^2/s at 40 °C	[20]
3	Density	DE	g/cm^3 at 20 °C	[20]
4	Cloud point	CP	°C (degrees Celsius)	[21]
5	Cold filter plugging point	CFPP	°C (degrees Celsius)	[22]
6	Bis-allylic position equivalents	BAE	mol/100 g oil	[23]
7	Allylic position equivalents	APE	mol/100 g oil	[23]
8	Long-chain saturated factor	LCSF	%	[22]
9	Higher heating value	HHV	MJ/kg	[20]
10	Saponification value	SV	mg KOH/g oil	[24]
11	Iodine value	IV	$g\ I_2/100\ g$ oil	[24]

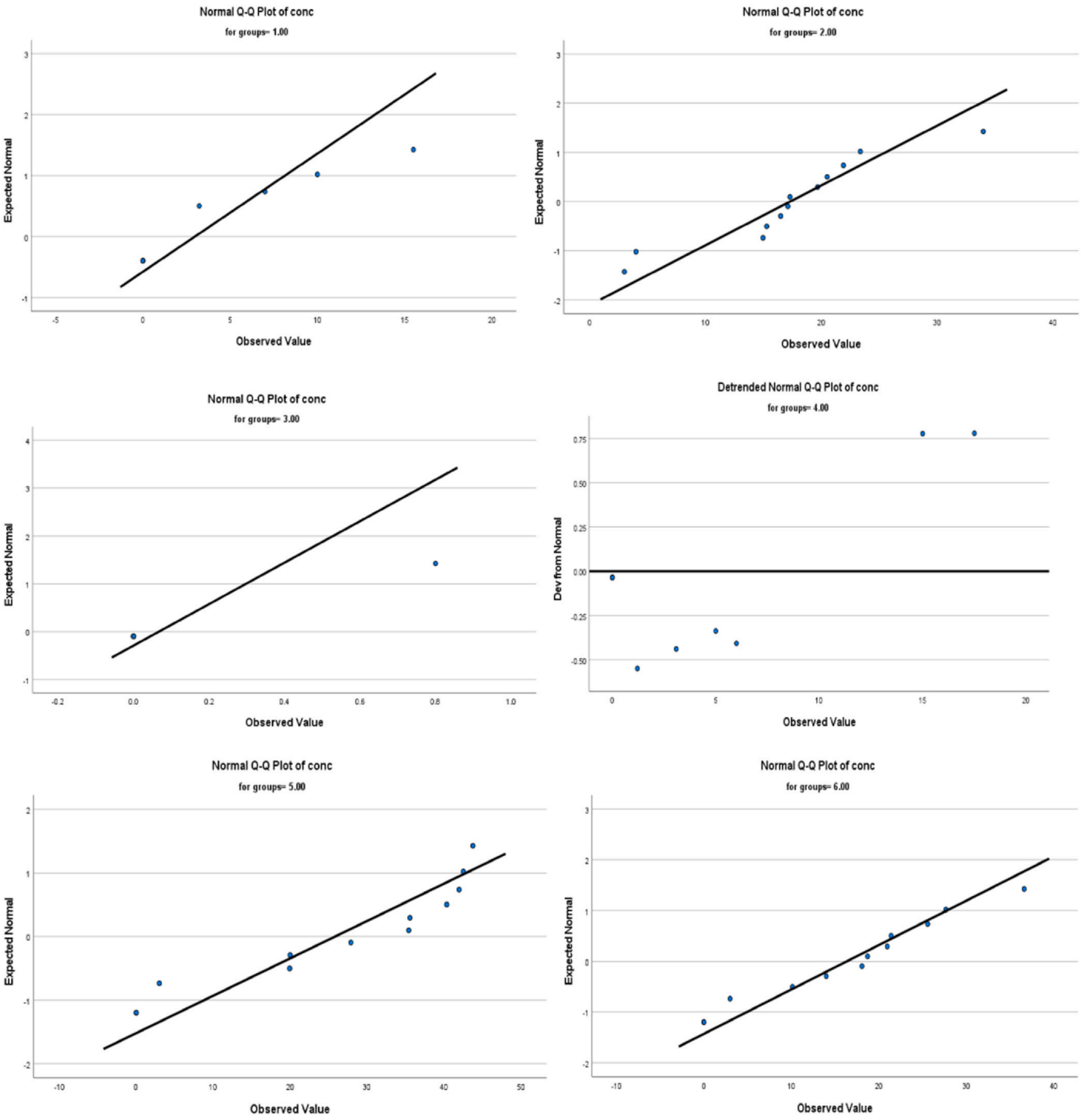

Fig. 1. The normal distribution analysis of FAMEs profile. FAMES = fatty acid methyl esters.

study on the importance of these kinds of FAMEs in the production of highly efficient biodiesel. However, there is an urgent need to find ways that would enhance the increase in the production of these types of FAMEs in a way that makes them more suitable for commercial purposes [29], one of the most valuable efforts in this field is modifying the metabolic activity of fungi to produce FAME, by using mechanisms to raise the concentrations of free fatty acids by deleting genes such as acetyl-CoA synthetase genes and an acyl-CoA oxidase gene [30]. While many studies have sought to grow fungi on sources derived from materials used as agricultural waste, which is considered an inexpensive source of carbon [31]. In the 2025 study by Abdelhamid et al. [32], 4 mutagenic agents were used to improve FAME production from fungi, the agents used were sodium azide, ethidium bromide, gamma radiation and ethyl methanesulfonate, which affected on C16 to C18 production, which are considered the most suitable for the esterification process. Furthermore, the production of biodiesel has good properties.

3.2. Cetane number

The properties, recital, and quality of biodiesel depend largely on the composition of the FAMEs it has. Procuring highly efficient biodiesel is neither easy nor economical. Therefore, studies have sought to pre-model its properties to determine the composition necessary to meet its requirements. CN is one of the most important properties of biodiesel fuels [33]. Biodiesel with good physical/chemical properties

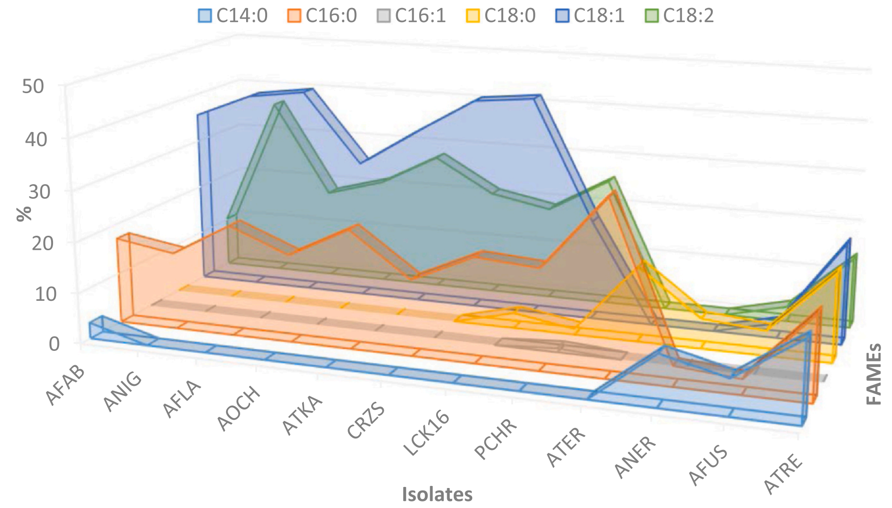

Fig. 2. The FAMEs profile of oleaginous fungi. FAMES = fatty acid methyl esters.

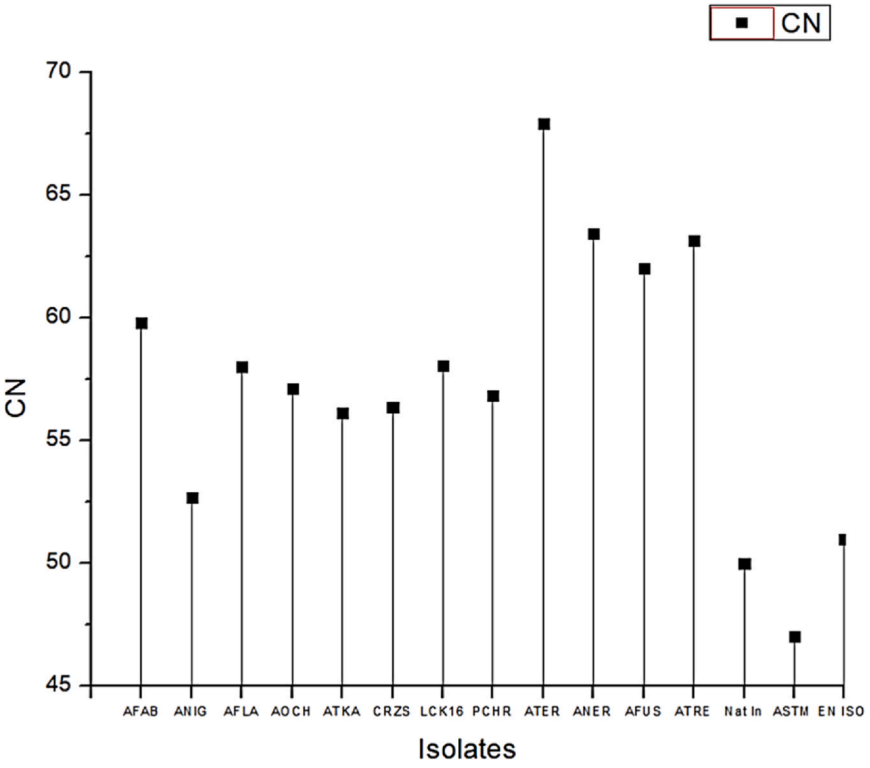

Fig. 3. The CN of Myco-diesel produced. CN = cetane numbers.

is crucial for achieving the highest quality diesel fuel, which positively impacts the fuel's efficiency in internal combustion engines. CN of approximately 44–60 are characteristic of highly efficient diesel [34]. These conclusions are in very good agreement with the results of our study, as the CN values of biodiesel produced from oleaginous fungi ranged from approximately 53 to 68, with a mode value of approximately 54 for most of the isolates. These numbers are in good agreement with ASTM, which prefers a CN for diesel of at least 47, as well as EN and the National Index (Fig. 3).

The distribution of unsaturated fatty acid (USFA) esters plays a very prominent role in determining the CN of biodiesel products. This relationship is clearly observed based on certain properties of these esters, for example, the percentage of double bonds (DB) or the ratio of monounsaturated fatty acids to SFA/USFA. Furthermore, an inverse relationship is observed between increasing fatty acid saturation and CN [33]. This view is very much in line with the results of the current study, which notes that the CN tends to increase linearly with the presence of low levels of USFA; this is most evident for both ATER and ANER isolates. Statistically, this relationship was clearly demonstrated after studying the linear relationship (regression analysis) between fatty acid group and CN levels. Built upon USFA and CN, it was found that there were clear statistical differences, as the P value reached 0.01 (Fig. 4). Similarly, the linear relationship between saturated fatty acid (SFA) levels and CN showed that there were statistical differences, with a P value of 0.047. As informed by FAME, prediction models for estimating the CN rate can be successfully and accurately used for biodiesel evaluation, providing a respected tool for reducing the time/fee linked with engine testing [35]. Ultimately, accurately predicting CN in addition to other biodiesel properties remains a persistent challenge due to the complexities of biodiesel combustion and the general dissatisfaction with finding a more acceptable/accurate indicator that provides results closer to reality, making these equations acceptable for

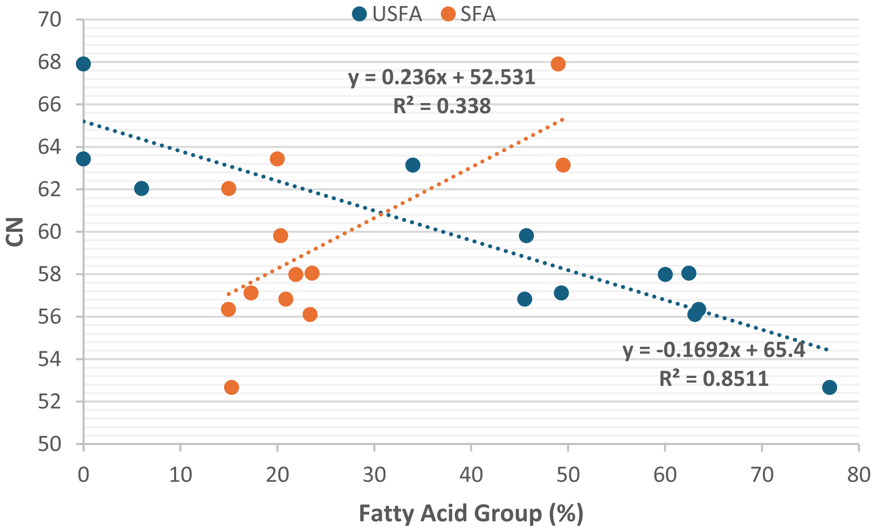

Fig. 4. The linear relationship between FAG (%) and CN. CN = cetane numbers; FAG = fatty acid group; SFA = saturated fatty acid; USFA = unsaturated fatty acid.

practical application. However, many researchers believe that the scientific facts derived from the equations for predicting the properties of biodiesel, identified by the number of cetane, represent realistic results that have proven to be effective [36,37].

3.3. Kinematic viscosity

To predict the behavior of injection, combustion systems in diesel engines, as well as to optimize these systems, studying the viscosity of biodiesel is of great importance [38]. Biodiesel viscosity can largely be attributed to the raw materials used in its production. The types of fatty acids used in biofuel production are among the most important factors that can positively or negatively affect biodiesel viscosity. Viscosity has a direct/significant impact on the quality of the fuel in various diesel engines. In any case, the viscosity grade of biodiesel must comply with the requirements of ASTM and EN [39]. Grounded in ASTM D445, at 40 °C, the kinematic viscosity (KV) should range from 1.9 to 6, with an average of 3.95 [25]. These results are consistent with the current study of diesel produced from oleaginous fungi, where the viscosity measured based on FAME content was very good considering ASTM D445 and EN ISO 3104 [12] (Fig. 5). In comparison with the fungal isolates, ANER/AFUS isolates showed viscosities of 1.16 and 1.21 mm^2/s, respectively, which are lower than the values approved by ASTM D445. The superiority of these isolates may be due to the low content of USFA, which are absent in both the C16:1, C18:1, C18:2 fatty acids of ANER isolate and the C16:1 fatty acid of AFUS isolate (Fig. 2). To prove this practically, these results led us to draw relationships between fatty acid content and KV, as well as to determine whether there is a linear relationship between them or not (Fig. 6). Considering this relationship, the results showed that there is a positive relationship between USFA-KV, which was clear in fungal isolates. In general, it can be concluded that increasing FAME increases KV, but the content of both USFA and SFA may affect these values. This opinion is completely consistent with both: Razali et al, (2024) [40] and Purwanto and Widianto [41].

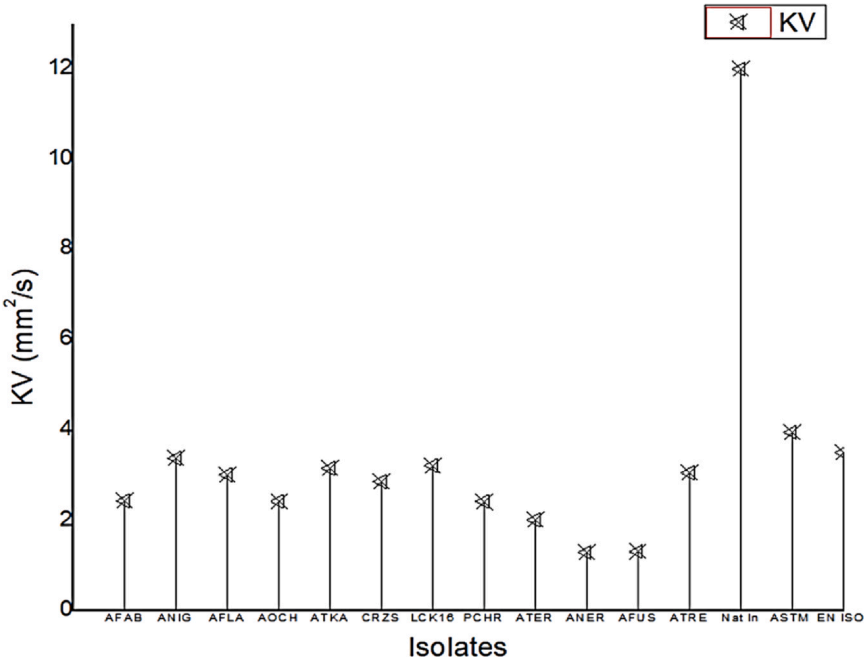

Fig. 5. The KV of myco-diesel produced. KV = kinematic viscosity.

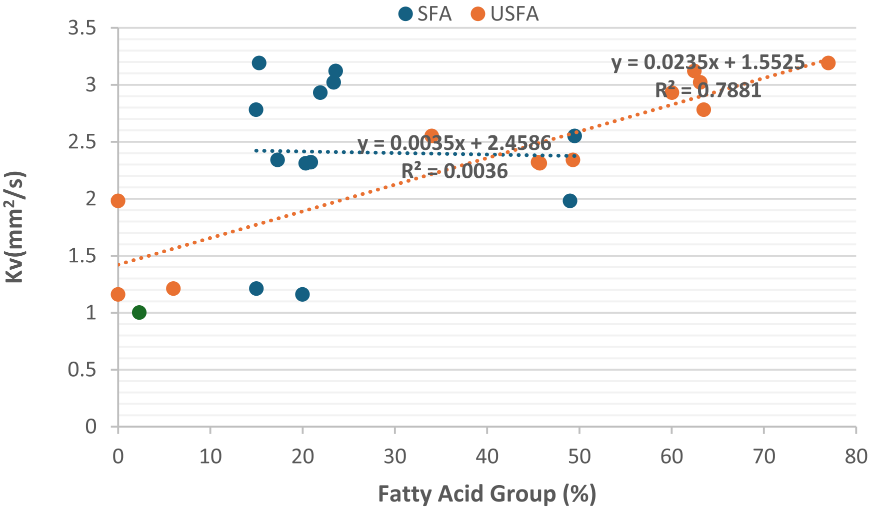

Fig. 6. The linear relationship between FAG (%) and KV. FAG = fatty acid group; KV = kinematic viscosity; SFA = saturated fatty acid; USFA = unsaturated fatty acid.

3.4. The density and cold flow properties

Based on FAME's structure, models were developed to predict biodiesel density, primarily based on the nature of the fatty acid esters that comprise it. As the number of carbon atoms increased, the density of SFA esters decreased at low temperatures, while the opposite trend occurred at high temperatures. These models yield very acceptable results for density prediction, despite the higher relative deviations, according to Ref. [42]. One of the most critical specifications for the volumetric/transport properties of biodiesel is that the density should be in the range of 860–900 kg m^{-3}[43], as density affects combustion engines through its impact on injector systems, as well as its impact on the CN [44]. Therefore, it is very important to pay attention to the volumetric/transport properties of biodiesel-fossil diesel blends when blending them together, paying attention to the atmospheric pressure criterion [45,46]. Derived from above, the studied oleaginous fungi demonstrated a density value (DE) that was largely consistent with both ASTM D6751–20a, EN ISO 3675 and slightly lower than the national standard in our country. These highly acceptable results are due to the esters of SFA/USFA, which resulted in a biofuel that met the above criteria, that was positively reflected in most of the distinctive properties of biodiesel, such as the CN (Fig. 7). Statistically, the linear regression relationship showed a positive relationship between the content of USFA/DE, which is clearly shown in Fig. 8. Moreover, the results showed that there is an inverse relationship between CN-DE, which is represented in Fig. 9. Achieving an acceptable level of density in biodiesel for engines is critical to improving combustion efficiency and reducing harmful emissions, in compliance with strict global warming in addition greenhouse gas emissions regulations. Recent research focuses on producing environmentally friendly, sustainable biodiesel that meets the important quality characteristics of biodiesel. Density is a key factor, as it plays a significant role in the ability of biodiesel to pass through filters, especially during low winter temperatures. This also affects its boiling point, which plays a significant role in its ability to start in cold conditions [47].

Despite all the advantages of biodiesel and the efforts to use it purely or blended with fossil diesel, it suffers from a fundamental

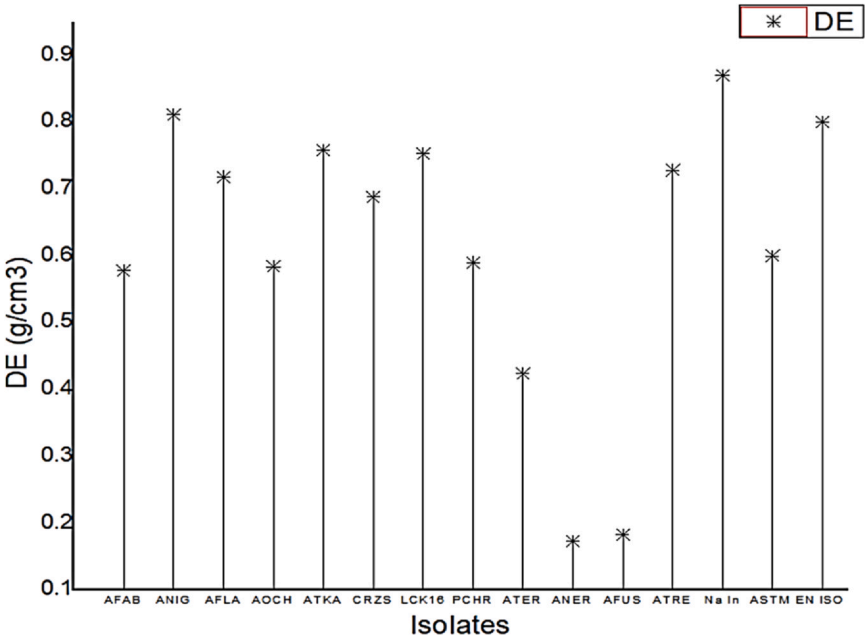

Fig. 7. The DE of myco-diesel produced. DE = density value.

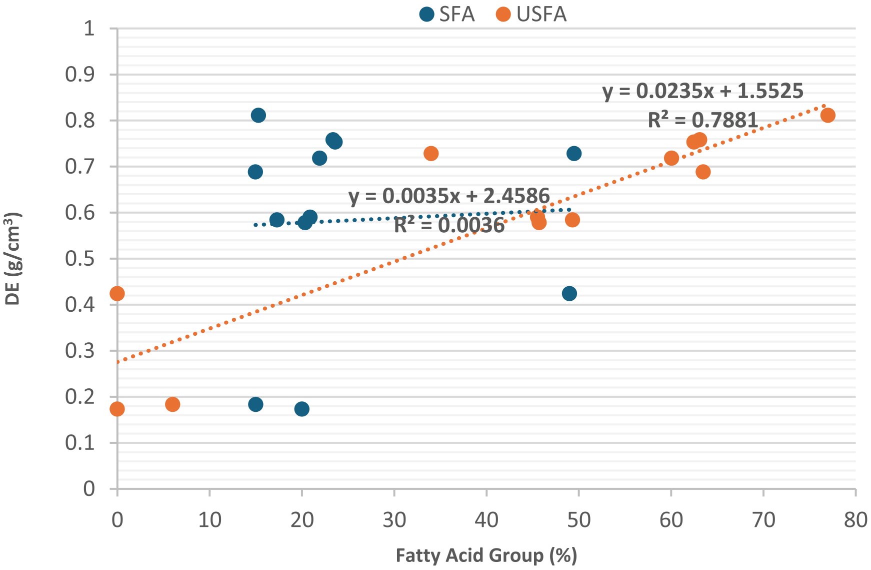

Fig. 8. The linear relationship between FAG (%) and DE. DE = density value; FAG = fatty acid group; SFA = saturated fatty acid; USFA = unsaturated fatty acid.

drawback in its cold flow characteristics (CFP). At low temperatures, the engine will not start due to filter clogging and the crystallization of fatty acids, the CP-CFPP are 2 of the most important parameters to be estimated to evaluate the cold flow properties. In winter, as temperatures drop, solid waxy crystalline nuclei form and grow, leading to what is known as CP [48]. Cold flow properties are of paramount importance to produce biodiesel that is acceptable in biodiesel engines. This interest is due to the problems that occur with this fuel in winter, which leads to the solidification of biodiesel in the fuel passages and the blockage of filters at low temperatures [49]. This factor is one of the most important factors affecting the quality of biodiesel, which has led to the restriction of its use in pure form to grade 100B [50]. My study demonstrated that most of the twelve targeted oleaginous fungi had optimal CP values as well as very acceptable CFPP values (Fig. 10). In temperate countries, the CP value is higher than in areas with low temperatures, the same applies to CFPP values. In comparison with the oleaginous fungal isolates, ATER/ATRE isolates had high values that might be unacceptable according to the standard biodiesel criteria. Given the nature of the fatty acid content of these isolates, this appears to be due to the presence of high concentrations of palmitic and stearic acid ethyl esters. The results of my study are in complete agreement with Bouaid et al. [48], who pointed out that there is a direct relationship between the decrease in both the palmitic/stearic acid ethyl esters content and decrease in the values of CP-CFPP. In complete contrast, looking at the relationships drawn in (Fig. 11), there is a clear inverse relationship between the content of USFA and the decrease in both CP and CFPP values.

To obtain biodiesel with acceptable values for CP-CFPP properties, many researchers have worked on various procedures to reach acceptable results. One of these procedures is the winterization process, which is a process used to remove waxy components/SFA from oils or biodiesel. This process uses the cooling property to improve the performance of biodiesel during cold weather periods [51]. Some researchers have worked on adding some materials to obtain acceptable results for both CP and CFPP. Leggieri et al. [52] improved the FAME by adding both applied dimethyl azelate and triacetin, which obtained acceptable results and also reduced the CP degrees by up to 3 °C. While Monirul et al. [53] added poly (methyl acrylate) to biodiesel, which resulted in a significant improvement in both the CP and CFPP values. One of the treatments used to overcome the problems of cold flow

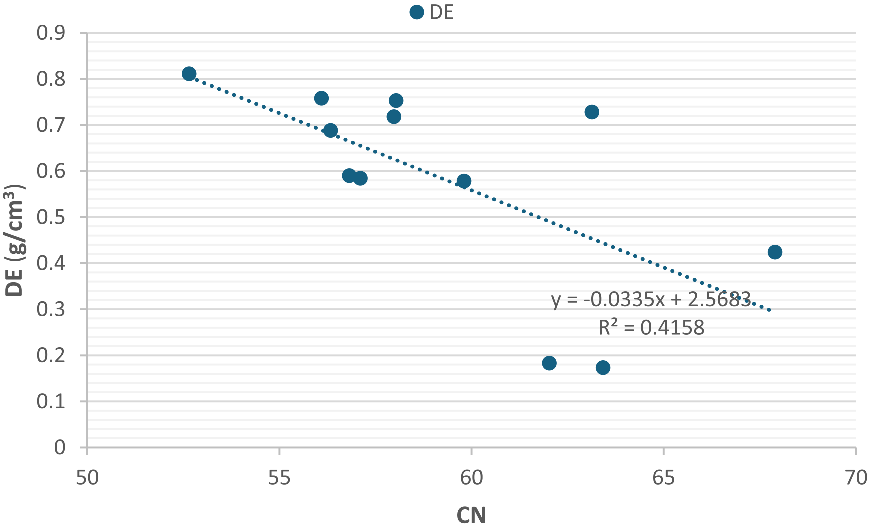

Fig. 9. The linear relationship between CN and DE. CN = cetane number; DE = density value.

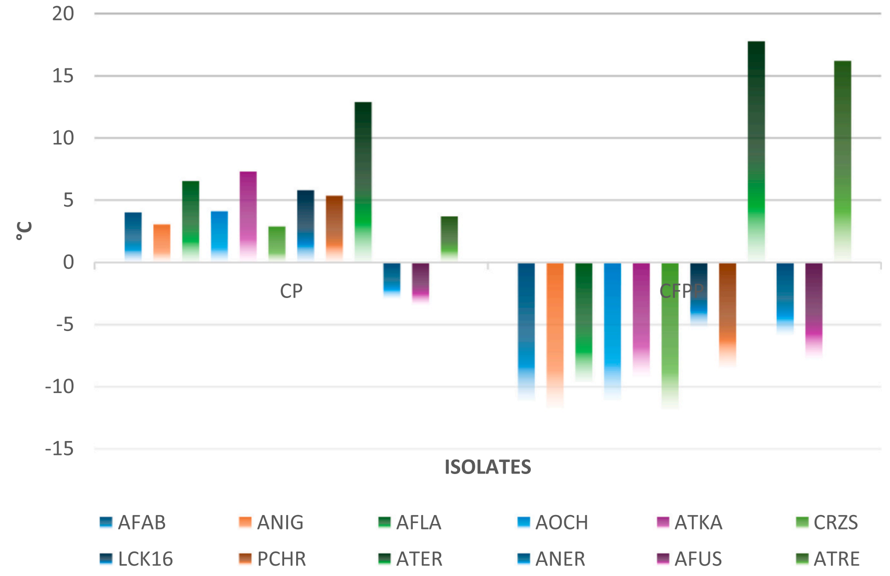

Fig. 10. The CP and CFPP of myco-diesel produced. CP = cloud point; CFPP = cold filter plugging point.

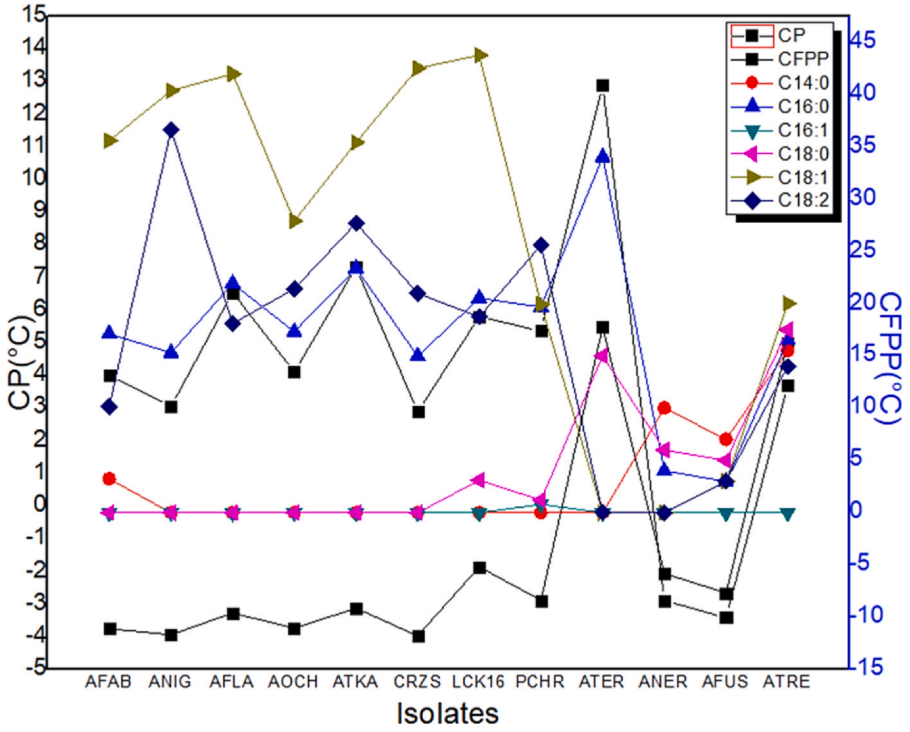

Fig. 11. The relationship between CP, CFPP and FAG profile. CP = cloud point; CFPP = cold filter plugging point; FAG = fatty acid group.

properties is the use of mixing or blending between bio/fossil diesel to suit internal combustion engines to obtain a premium fuel that can overcome the problems [54].

3.5. Bis-allylic/allylic position equivalents

Biodiesel is produced from the methyl esters of long-chain fatty acids using oils and fats from a variety of sources. Commercially, biodiesel is produced by the esterification of triglycerides, a process that uses basic/acidic catalysts and methanol/ethanol. The produced biodiesel undergoes auto-oxidation, which negatively impacts its quality in addition to storage. This oxidation renders it incompatible with internal combustion engines. Therefore, oxidation is a critical process that can influence whether biodiesel is accepted or rejected [55]. Oxidative stability of biodiesel is a critical issue, as it affects the properties of biodiesel fuel, which directly impacts engine performance. Both physical/chemical factors play a pivotal role in oxidative stability, a highly complex property influenced by multiple factors [56]. Such as minerals, light, heat, air, peroxides, antioxidants, etc. Furthermore, biodiesel can be more susceptible to moisture saturation than fossil diesel, which leads to hydrolysis, resulting in fatty acids. Furthermore, the literature indicates that the chemical composition of biodiesel is the main factor for autoxidation. However, the nature of autoxidation in biodiesel is not fully understood, so many authors attribute this process to the fatty acid composition-structure [57]. Therefore, it is necessary to understand the effects separately to build a sound scientific understanding of the causes and roles of each factor in the oxidative stability of biodiesel[56]. Built upon fatty acid esters produced from oleaginous fungi, my study showed that there is a strong positive relationship between the concentration of USFA, Bis-allylic position equivalents (BAE) and allylic

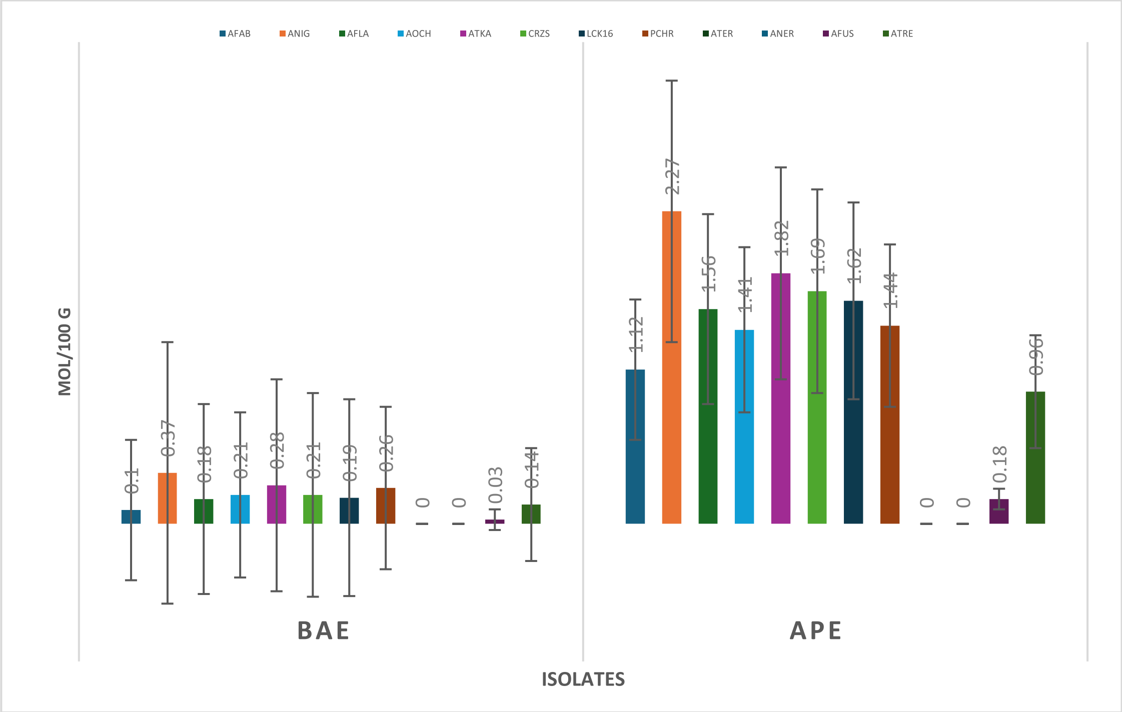

Fig. 12. The BAE and APE of Myco-diesel produced. APE = allylic position equivalents.

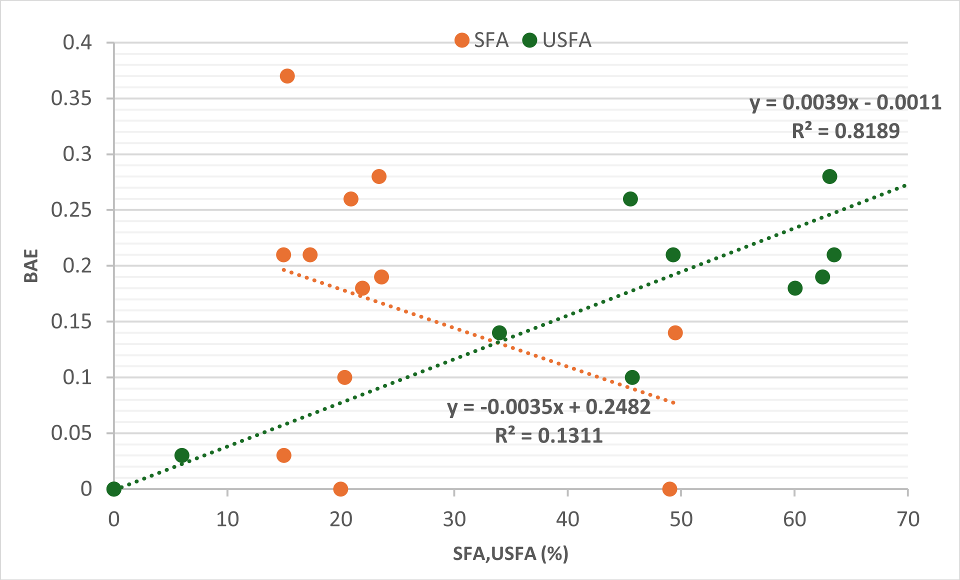

Fig. 13. The linear relationship between SFA, USFA and BAE. SFA = saturated fatty acid; USFA = unsaturated fatty acid.

position equivalents (APE), with $R^2 = 0.8189$ and $R^2 = 0.9826$, respectively. In contrast, the study showed that there is an inverse relationship between BAE-APE depending on the content of SFA (Figs. 12–14) while, showed that there is a strong positive correlation between BAE- APE (Fig. 15). Largely due to the high concentrations of USFA in oleaginous fungi, Myco-diesel can be susceptible to oxidation, thus affecting the oxidative stability and storage periods of this type of fuel. Although this problem can be solved by using the additives or using it in a mixed form < B100, it also contains excellent concentrations of saturated fats, which can significantly improve the oxidative stability of Myco-diesel. This is more clearly observed when calculating the long-chain saturated factor of oleaginous fungi. The study showed that 10 out of 12 fungal isolates had a level of long-chain SFA that was largely acceptable (Fig. 16), which inevitably affects the oxidative stability of Myco-diesel, in addition to its impact on the freezing point and flow characteristics at low temperatures. This oxidation process ultimately increases the viscosity of biodiesel and creates harmful deposits that affect its operation. Consequently, it also causes long-term instability when stored in tanks. Therefore, synthetic antioxidants are typically used to address this problem [58]. Ultimately, the problem of oxidative stability of biodiesel remains despite its great advantages.

3.6. *The higher heating value*

The resumption of the use of biomass-derived biofuels in the 21st century is linked to the declining fossil fuel reserves compared to other types of resources, as well as to mitigating their harmful impact on the climate and environment. The primary quantitative property of fuels is

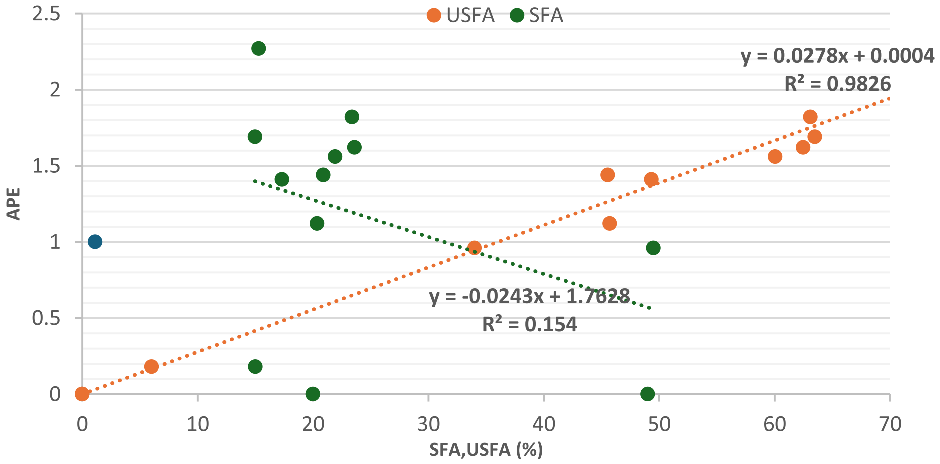

Fig. 14. The linear relationship between SFA, USFA, and APE. APE = allylic position equivalents; SFA = saturated fatty acid; USFA = unsaturated fatty acid.

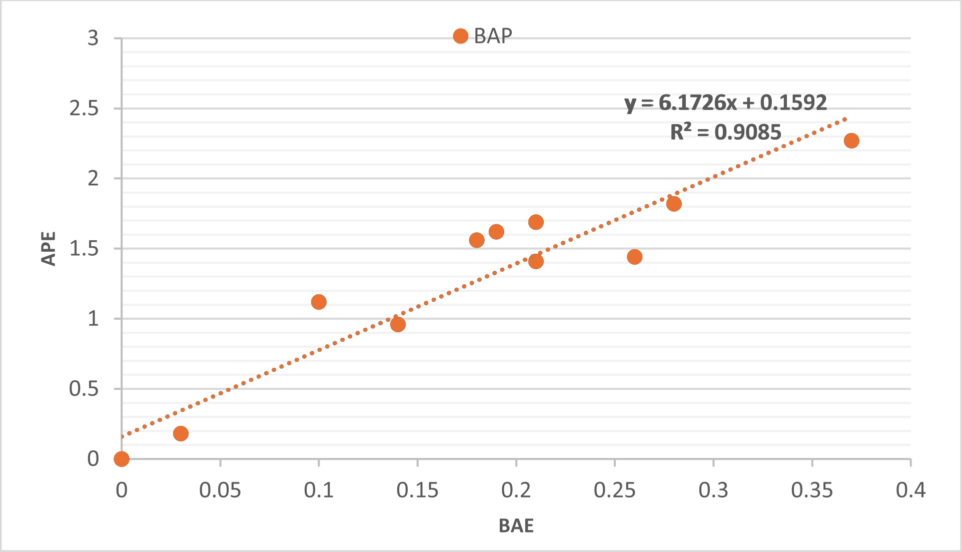

Fig. 15. The linear relationship between APE and BAE. APE = allylic position equivalents.

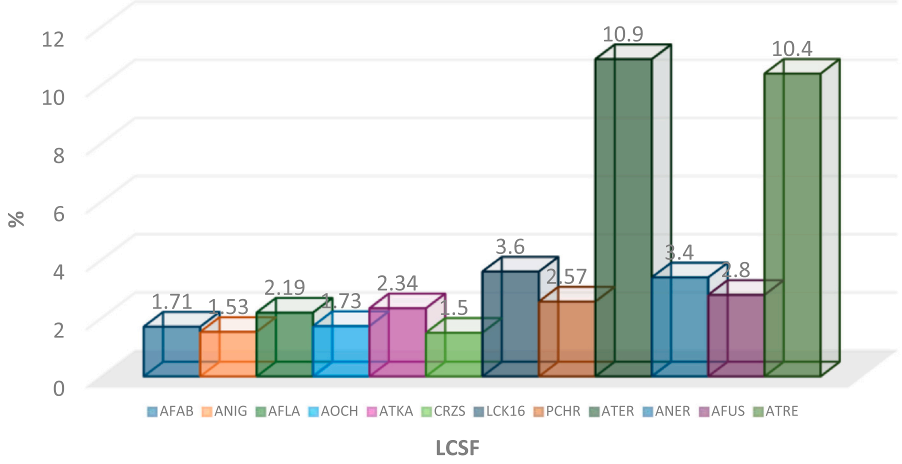

Fig. 16. The LCSF of Myco-diesel produced. LCSF = long-chain saturated factor.

their calorific value [59]. One of the most important properties of biofuels is the calorific value calculations in numerical simulations of biomass thermal conversion systems [60]. The calorific value of a fuel is the heat of combustion [61], which is the energy released during fuel combustion. Fuels with a higher calorific value are preferred because they are considered more efficient for small-scale engines [62]. The higher heating value (HHV) value increases with the increase in the number of carbon atoms (C) in the FAME; the relationship between the ratio of carbon-hydrogen to oxygen-nitrogen is an essential relationship in the HHV value [20]. The results of my study are fully consistent with

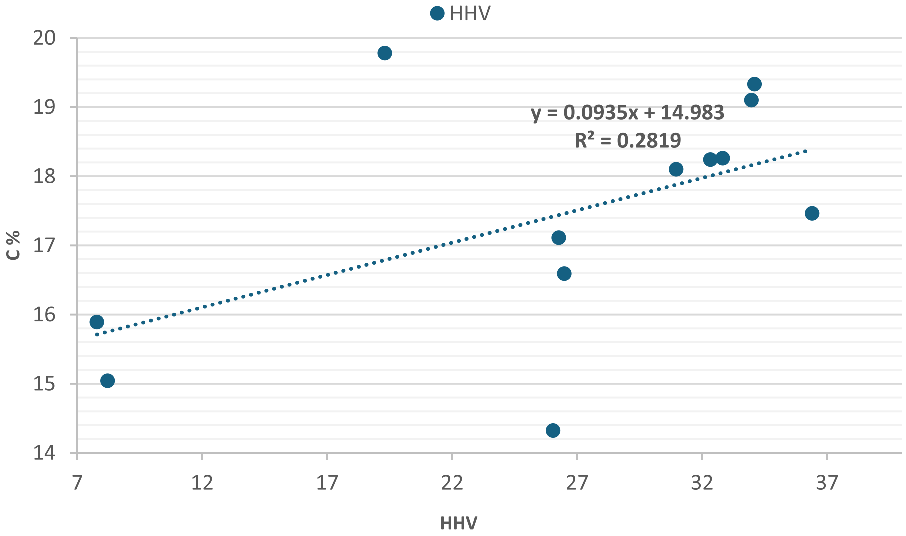

Fig. 17. The linear relationship between C and HHV. HHV = higher heating value.

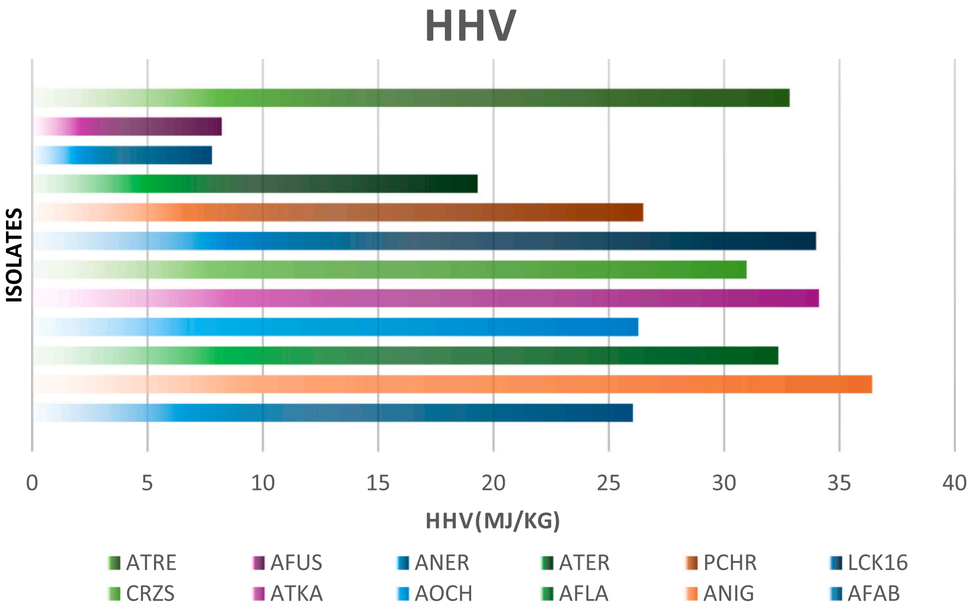

Fig. 18. The HHV of myco-diesel produced. HHV = higher heating value.

this view, as the correlation between the number of carbon atoms and the HHV value is positive, as clearly shown in Fig. 17. Furthermore, the results of the targeted oleaginous fungi in the study showed HHV values exceeding 20 in most isolates; they did not reach 40, while there were isolates that did not exceed 20, and these percentages are considered unacceptable according to standard criteria [63]. However, they fell below 10 in ANER/AFUS isolates (Fig. 18). The researchers have been interested in finding different prediction models to calculate the calorific value, whether low or high, as calorific values affect engines directly in several aspects, the most important of which is its impact on fuel consumption in addition to achieving thermal efficiency. The high calorific value can affect the density of biofuel or viscosity, as it can affect the CN, iodine value (IV), and other effects [64]. In general, the high calorific value of diesel fuel ranges between 45 and 46 MJ/kg, compared to biodiesel, which shows values up to about 40 MJ/kg. Based on the data presented in my study, Myco-diesel has lower energy values than fossil diesel. This may be since FAME molecules contain oxygen atoms embedded in their structure. This means that a portion of the mass is not used to generate heat energy but rather enters directly into the reaction. Compared to petroleum diesel, which consists only of hydrocarbons (C / H), it is more energy concentrated. This view is consistent with Waanders et al. [65], in their comparative study of the 2 types of fuel.

3.7. The saponification and iodine values

The saponification value (SV) is usually determined by a method known as acid-base titration. The IV is commonly used to evaluate the grade of unsaturation of lipids/fats. This determination is informed by the reaction of DB in the interior fatty acids with monoiodine bromide. This reaction involves various steps and is time-consuming [66,67]. SV is one of the most important indicators of oil quality. It can be defined as the amount of alkali, which is always expressed in mg KOH/g oil, required to saponify an oil sample. This amount is usually determined by estimating the alkali required to saponify long-chain fatty acids. A low SV usually indicates that triacylglycerols contain abundant amounts of long-chain fatty acids, as the moles of base reactant are equivalent to the moles of long-chain fatty acids. Conversely, a high SV indicates the presence of short-chain fatty acids in the triacylglycerols [68]. Based on the above, the balance between long/short-chain fatty

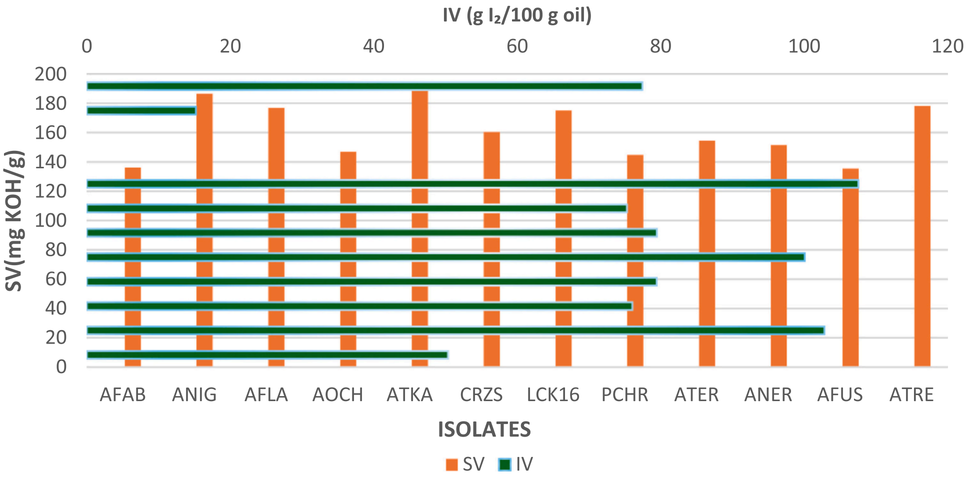

Fig. 19. The SV and IV of Myco-diesel produced. IV = iodine value; SV = saponification value.

acids in fungal lipids is crucial for achieving optimal SVs. This is clear for the oleaginous fungi studied, as the SVs for most isolates were > 100 - < 200 mg KOH/g oil (Fig. 19). The SVs of oleaginous fungi are consistent with those of animal-vegetable lipids. For example, rapeseed, sunflower, and soybean oils, which contain long-chain fatty acids such as C18 and C16, have similar SV values ranging from 168 to 196 mg KOH/g oil [69]. Coconut and palm kernel oils, which contain large amounts of C12:0/C14:0 fatty acids, have much higher SVs of 235–260 [70,71].

As informed by EN, EN 14214 [10], the diesel produced from fungi showed optimum values, as all twelve isolates studied did not exceed 120 g I_2/100 g (Fig. 19). The IV is among the most important properties studied for biodiesel. This value depends on the composition of the unsaturated methyl esters in biodiesel. These values directly affect oxidative stability [72] and filter clogging in cold conditions [73]. IV represents the mass of iodine absorbed in 100 g of biodiesel through the interaction between carbon-carbon DB and I_2, ultimately providing a measure of the degree of unsaturation of the esters [73]. The interaction of these unsaturated esters with oxygen can lead to the formation of primary oxidation products, which in turn react with secondary oxidation products. The most important primary oxidation products produced are hydroperoxides and peroxides [74]. These reactions ultimately lead to the degradation of biofuels as well as the formation of undesirable residues. This degradation inevitably affects both the CN and other properties of biofuels[75].

4. Techno-economic assessment and future perspective

Because microorganisms, including fungi, can accumulate lipids within their biomass, researchers are pursuing ambitious research goals to find alternatives to fossil fuels, which suffer from numerous drawbacks, such as pollution from their combustion and their limited availability in certain regions. Esters of fatty acids produced by oily fungi are among the most promising sources for producing biodiesel derived from the esterification of their oils. The advantage of these esters lies in their close similarity to esters derived from plants from which biodiesel has already been produced commercially [29]. Biodiesel can gain acceptance and compete with other sources if it meets international standards for biodiesel [76], which partially aligns with analyses conducted on the produced diesel, showing increased quantities of methyl esters of unique fatty acids and achieving specific quality levels for the esters produced after the esterification process [30].

To achieve goals related to the fourth generation, researchers sought to improve and develop the production of fatty esters by these organisms through processes that included deleting or modifying genes such as acetyl-CoA genes[31]. Researchers also achieved other positive results by using chemical agents such as sodium azide and ethidium bromide, and physical agents such as gamma rays, with the aim of improving and achieving qualitative development at the level of the fatty acids (FAMEs) produced by these organisms[32]. Their research effectively resulted in achieving acceptable levels of increased levels of certain fatty acids that clearly affect the quality of the produced biodiesel, especially the C16 to C18 types [33]. This production is linked to achieving a balance between the quantitative production of biomass and the qualitative value of these esters, relying on sources that are not only inexpensive or low-cost [32]. Therefore, the production of biodiesel from fungi based on biomass and fungal lipids must achieve goals that are not only related to quantity and quality, but also extend to the possibility of producing it cheaply, which can make it truly competitive with other sources [8].

Fungal-derived fatty acid esters are likely to play a pivotal role in improving the properties of biodiesel. However, this concept is theoretically and practically linked to the biodiesel produced and its acceptability according to international standards. For example, both the chemical and physical properties of fungal diesel must be compatible with internal combustion engines without modifications that could increase costs[8]. The CN is one of the most important of these properties [35]. According to the American Society for Testing and Materials (ASTM), the acceptable CN should be at least 47. This is consistent with the results of the current study and has already been achieved with the fungal isolates studied and other studies of oleaginous fungi[34]. The quality of biodiesel is also primarily related to its viscosity[39], which should not exceed 6 at 40 °C according to ASTM D445 [40].

To reduce density in practice, blending biodiesel and petroleum diesel in varying ratios is commonly employed. This is particularly suitable for internal combustion engines, as this property significantly impacts transportation and fuel combustion. Generally, a balanced distribution of USFA and SFA esters can produce biodiesel with balanced density characteristics. Blending fossil diesel and biodiesel can achieve a density of 860–900 kg/m^3, according to[44–46]. The importance of these parameters lies in ensuring biodiesel can pass through filters without solidification, which can occur in low-temperature environments[48]. Furthermore, the fatty acid balance results in higher saponification and IVs [77], as well as HHVs [21] and better cold flow properties[53]

Considering all these parameters is crucial for achieving the distinctive characteristics of the produced biodiesel. However, production processes remain a significant obstacle to achieving biodiesel production that can compete with other sources. These obstacles are related to several factors, the most important of which is the rapid production of good-quality biomass using inexpensive media [78]. Fermentation conditions and the time required to produce this biomass also play crucial roles in achieving these objectives [79]. My study has shown that not all fungal isolates are capable of achieving the optimal levels associated with high-quality biodiesel production. Furthermore, although some fungal isolates have achieved more acceptable results than others, some parameters or measurements remain less than ideal compared to international biodiesel standards. It can be said that achieving biodiesel based on the biomass of fungi is only in the research stages, and this aspect needs more studies through which higher-yielding isolates or those more capable of utilizing cheap carbon sources must be linked to the production of fatty acids balanced between all saturated and USFA, which will be reflected positively in achieving acceptable results and properties for biodiesel.

5. Conclusion

Given the urgent need for clean and promising energy sources globally, oleaginous fungi demonstrate good lipid accumulation capabilities that can be extracted and utilized in biodiesel production. Although commercial production has not yet been implemented, some fungal isolates have shown promising results in producing FAMEs that meet ASTM/EN ISO standards. However, its production requires the use of different techniques/methods to maximize the production of effective FAMEs, making it a superior biodiesel source while overcoming the risk of oxidation within short timeframes. On the other hand, while many properties related to the FAME content produced by several fungal isolates are ideal, some isolates, such as ANER/AFUS, have failed to achieve high quality based on FAMEs produced after esterification. From my perspective that there is a pressing need for other isolates from other environments that can achieve high biomass, greater fat accumulation, and be cheap and economical, in addition to the possibility of introducing aspects of technical engineering in enhancing aspects of production and moving from the stage of theorizing to the stage of implementation and production, which moves the work from restricted and limited work to work that can be verified according to reliable laboratory measurements, which remains a goal in order to achieve new sources that compete with the sources currently used.

CRediT authorship contribution statement

Kadhim Fadhil Kadhim: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

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Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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