



Optimization of Heterogeneous Transesterification of Non-Food Sources To Produce Biodiesel

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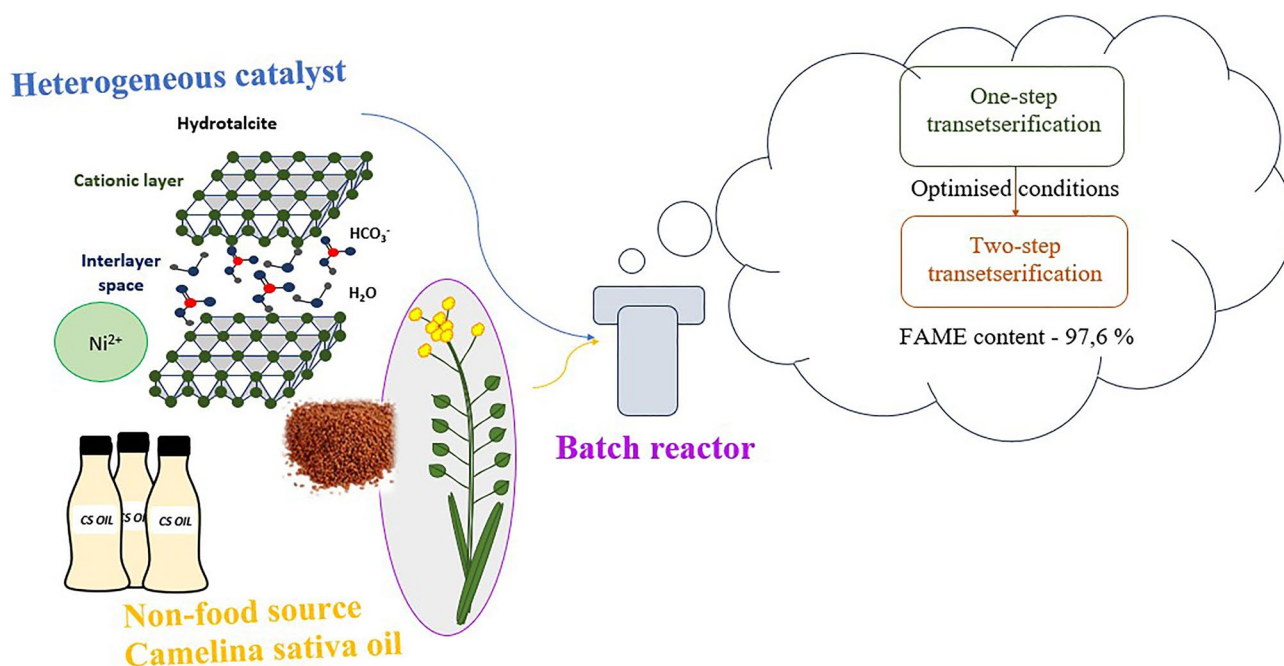
Abstract

The paper is focused on the optimization of reaction conditions to produce biodiesel by heterogeneous transesterification over Ni-Mg/Al mixed oxide. Preparation of biodiesel by the commonly used homogeneous transesterification produces a large amount of wastewater and other disadvantages like soap formation, difficult separation, polluted glycerin, and limitation of the catalyst used due to the content of free fatty acids. Heterogeneous transesterification offers easier separation and the possibility of reusing catalysts. As an oil source of vegetable oil, non-food plant *Camelina sativa* was used. Ni-Mg/Al mixed oxide was obtained from hydrotalcite, prepared by co-precipitation. The catalyst was characterized by different techniques to measure basicity, acidity, crystalline structure, and textural properties. The novelty lies in the complex optimization of reaction conditions (ratio of methanol to oil, amount of catalyst to oil, reaction temperature, reaction pressure, reaction time) to obtain a high content of FAME in biodiesel due to the European standard and in the introduction of two-step transesterification from an unconventional source. Transesterification was carried out in a batch reactor. Optimal reaction conditions to obtain the content of FAME in biodiesel higher than 96.4 wt% were the reaction temperature of 140 °C, for 7 h with a molar ratio of methanol to oil of 18/1, an amount of catalyst 3 wt%, and a pressure before reaction of 0.5 MPa(g) for one-step transesterification. For two-step transesterification, two reactions for 3 h at 140 °C, a molar ratio of 18/1, and 3 wt% of catalyst reached FAME content more than 97%.

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Graphical Abstract



Keywords Hydrotalcite · Biodiesel · Transesterification · FAME · Catalyst

1 Introduction

In recent years, fossil fuel consumption has significantly increased, largely due to heightened demand in various industries, with transportation being a major contributor. As global living standards improve, the number of vehicles on the roads is also rising, leading to greater demand for fuel [1, 2]. However, this trend is linked to environmental challenges, primarily because of the release of greenhouse gases (GHGs). The transportation industry is the third-largest contributor to emissions, after the industrial and construction sectors [3, 4]. Demand is expected to grow further in the coming years, accompanied by a corresponding increase in greenhouse gas emissions [5]. Consequently, ongoing efforts are focused on developing alternative methods to replace fossil fuels across different industries. A particular emphasis is placed on biodiesel as a promising substitute for conventional diesel in engine applications.

Currently, biodiesel is mainly used as a blending component with diesel fuel, with its concentration typically varying between 7 and 20% depending on the country [6]. Although biodiesel production has become an established technology in recent years, several challenges in its synthesis still need to be tackled. These include concerns related to the raw materials used for production, as well as the processes and catalytic systems involved in the reaction, which continue

to be the focus of research worldwide [7–10]. Biodiesel is most commonly produced through a transesterification reaction, where triglycerides react with an alcohol to generate fatty acid methyl esters (FAME). The process requires the presence of a catalyst to proceed efficiently. A key challenge in biodiesel manufacturing lies in sourcing triglycerides; vegetable oils are frequently used due to their availability, favourable physicochemical properties, and high triglyceride content [11]. However, the predominant oils, such as rapeseed and sunflower, are primarily edible, raising concerns about using food resources for fuel production [12]. As a result, current research efforts are directed toward identifying alternative feedstocks, including non-food oils, and used cooking oils [9, 13]. From unconventional sources for biodiesel production, microalgae, fish fats or cyanobacteria are studied [14]. As an alcohol for transesterification, methanol is most commonly used. After transesterification unreacted methanol is recovered usually by distillation or other energy efficient processes [15].

One of the oils explored for transesterification is derived from *Camelina sativa*. This plant can be cultivated as either a summer or winter crop across diverse climatic regions [16]. The oil yield from its seeds is comparable to that of widely used oils like rapeseed and sunflower. An advantage of *Camelina sativa* oil is that it is not typically used for food, and its cultivation requires fewer pesticides and fertilizers compared to other common crops. However, a notable

drawback is its high iodine value, indicating a high content of unsaturated bonds in the oil [17, 18]. Before using it as an oil for transesterification commonly used refining process, which is used for sunflower or rapeseed oil, could be used [19].

An important consideration in biodiesel production is the choice of catalyst. Currently, homogeneous catalysis is the most widely used method [20]. Homogeneous catalysts, such as sodium or potassium methoxide, pose significant environmental challenges. The primary issue is the difficulty in separating the catalyst from the biodiesel and glycerol products [21]. This separation typically involves extensive washing with large volumes of water, resulting in considerable wastewater generation. Furthermore, homogeneous catalysts are sensitive to free fatty acids and water content, which can impair their effectiveness. Additionally, these catalysts cannot be regenerated or reused, limiting their sustainability [22]. Consequently, research is shifting focus toward heterogeneous catalysis. Solid catalysts—including oxides, mixed oxides, hydrotalcites, and other materials—have demonstrated promising results, achieving high biodiesel yields [23, 24]. These catalysts offer advantages such as the potential for regeneration and reuse, as well as eliminating the need for extensive washing. However, they also present challenges, including longer reaction times and the requirement for higher temperatures and pressures. Addressing these issues remains a key goal of ongoing research efforts. There are different types of heterogeneous catalyst, that are currently studied for biodiesel preparation, for example metal-organic frameworks of hydrotalcites [25].

Hydrotalcites, with their layered structure and simple synthesis, appear to be suitable precursors for mixed oxides used as catalysts in biodiesel production [7]. Thanks to the possibility of embedding various metals into their structure, it is possible to alter their physicochemical properties and thereby achieve higher catalyst activity and stability during the reaction [22]. Through calcination, a structure composed of mixed oxides can be obtained, which exhibits high activity in transesterification reactions [26].

The novelty lies in the optimization of reaction conditions for heterogeneous transesterification on a non-food, unconventional camelina sativa oil using mixed oxides with incorporated nickel into the structure of basic Mg-Al hydrotalcite. An integral part of the optimization is the implementation of a two-step transesterification process to achieve the minimum required FAME content, which is more than 96.5% by weight.

2 Materials and Methods

2.1 Catalyst Preparation

Hydrotalcite was prepared in one batch for all transesterification to ensure the same catalyst properties. The co-precipitation method with a constant pH was used for hydrotalcite preparation. Three different solutions for precipitation were prepared: (1) the nitrate solution (1 mol/dm³) containing nitrates (Mg(NO₃)₂, Al(NO₃)₃, Ni(NO₃)₂) as a source of cations; (2) the solution of NaHCO₃ (1 mol/dm³); (3) the solution of NaOH (3 mol/dm³) for holding pH on a constant value. Solution 1 was slowly added to solution 2 for one hour under intensive stirring by a peristaltic pump. The addition of solution 3 was controlled by a pH value. After precipitation, hydrotalcite was aged for 24 h and then washed out to obtain a neutral pH of the washing water. Overnight at a temperature of 80 °C, hydrotalcite was dried. Before transesterification, the mixed oxides structure was made by calcination at a temperature of 450 °C.

2.2 Catalyst Characterization

XRD patterns of mixed oxide structure (obtained from hydrotalcite by calcination at a temperature of 450 °C before measurement) and hydrotalcite crystalline structure were measured by diffractometer (STOE Theta/Theta diffractometer) with Co K α . A scan range of 2.9–90.00°2 θ and a step size of 0.02° were used.

Textural properties (specific surface – SBET, pore Volume – V_p) were measured by physical adsorption of nitrogen at a temperature of liquid nitrogen (–195 °C) (Micromeritics, ASAP2400). Data were evaluated by the BJH method and BET isotherm. The volumetric method to measure the adsorption-desorption isotherms was chosen. The measurements were performed in the relative pressure range p/p₀=0.01–0.995 (adsorption) and p/p₀=0.995–0.2 (desorption). The saturated vapor pressure was recorded every 120 min. From the volume of adsorbed nitrogen at a relative pressure of 0.99 (V_a), we determined the total pore volume V_p and converted it to liquid nitrogen according to the following equation:

$$V_p = V_a \cdot 0,00155$$

When calculating the specific surface area, the BET isotherm in the range p/p₀ ∈ <0.05–0.25> was used. The specific surface area from the slope and intercept of the BET isotherm was calculated. By linearizing the BET isotherm for microporous materials, negative intercept of the straight line equation was obtained. The specific surface area was calculated based on the following relationship:

$$S_{BET} = \frac{4,353}{a + b}$$

The acidity and basicity of the mixed oxide were determined by temperature-programmed desorption of CO₂/NH₃. A detailed description of the measurement at Malisova M. et al. [27].

The dependence of sample mass on increasing temperature (thermogravimetry) was measured using a PerkinElmer TGA 4000 device. The temperature rise during sample measurement was set at 10 °C/min within the range of 30 to 600 °C. Before measurement, an exact amount of the sample was weighed onto a ceramic sample holder using analytical balances and carefully placed on the device's weighing pan with tweezers. After recording the initial weight of the sample with the instrument, heating was initiated, and the changes were monitored and recorded.

SEM scans were measured as powder samples calcined at a temperature of 450 °C. Measurements were taken using the SEM-TESLABS-300 instrument in conjunction with the TESCAN digitization unit. The recording and its evaluation were performed using the WinTIP v3.1 software.

2.3 Camelina Sativa Oil

Camelina sativa var. Smilnowska plant was cultivated at the National Agricultural and Food Centre in the Slovak Republic. Oil was obtained by cold pressing at the organization of Energy 21. Before transesterification, Camelina sativa oil was raffinate by the refining process [28].

2.4 Heterogeneous Transesterification

The transesterification reaction was carried out in a batch reactor (Parr Instruments, model 4520) made of nickel-chromium steel with maximum volume of reactor 200 ml. Stirring was provided by a magnetic stirrer for all reactions at a rate of 500 rpm. The reactor was heated by the heating ring to the required temperature.

2.4.1 One-step Heterogeneous Transesterification

Reaction conditions varied based on the studied reaction conditions (time, temperature, molar ratio of oil to methanol, amount of catalyst to amount of oil, pressure). Always, only one reaction condition was changed, and the others were the same. After the reaction catalyst was separated from the liquid products. Unreacted methanol was evaporated by a vacuum evaporator. Glycerol was separated by centrifugation.

2.4.2 Two-Step Transesterification

The procedure consisted of two sequential steps, both performed under identical optimised conditions from one-step transesterification (molar ratio methanol/oil 18:1, amount of catalyst 3 wt% to weight of oil, temperature of 140 °C, pressure before reaction 0,5 MPA(g)). Reaction time varies in each test. Initially, dried methanol, refined oil, and calcined catalyst were combined and subjected to the first step of the reaction. After the first step, the process was stopped, unreacted methanol was separated, and glycerol was removed from the mixture of unreacted oil and biodiesel. The remaining mixture, comprising unreacted oil and FAME, was then transferred to the second step. In this phase, fresh methanol and the fresh catalyst were added to the mixture. The second transesterification also took place at optimized conditions for the second step. Post-reaction, excess methanol was eliminated through vacuum evaporation.

2.5 FAME Content Analysis

Fatty acid methyl ester content in biodiesel was determined by GC with a flame ionization detector (Agilent 7890 A series with split/splitless injector) based on standard EN 14,103. Agilent technologies column BD-EN 14,103 with dimensions 30mx0.32 mm and 0.25 µm film thickness was used. As the internal standard, the nonadecanoic acid methyl ester was used. Final content was calculated based on peak area and weights of the standard and sample.

3 Results and Discussion

3.1 Catalyst Characterisation

For the optimization of heterogeneous transesterification in one-step and two-step transesterification, a Ni/HT mixed oxide prepared from hydrotalcite structure was used, obtained by calcination at 450 °C. The hydrotalcite structure was prepared via co-precipitation with nickel incorporated into the hydrotalcite structure during the precipitation process. The sample of hydrotalcite and the prepared mixed oxide were characterized to obtain their physicochemical properties.

Using XRD, the structural properties of the prepared sample were measured. In Fig. 1, the XRD patterns of the hydrotalcite structure (A) and the mixed oxide structure (B) are visible. In the hydrotalcite structure, characteristic absorption bands corresponding to the crystalline structure of hydrotalcite can be seen. XRD confirmed that the incorporation of nickel into the hydrotalcite structure did not change the typical hydrotalcite structure. During calcination

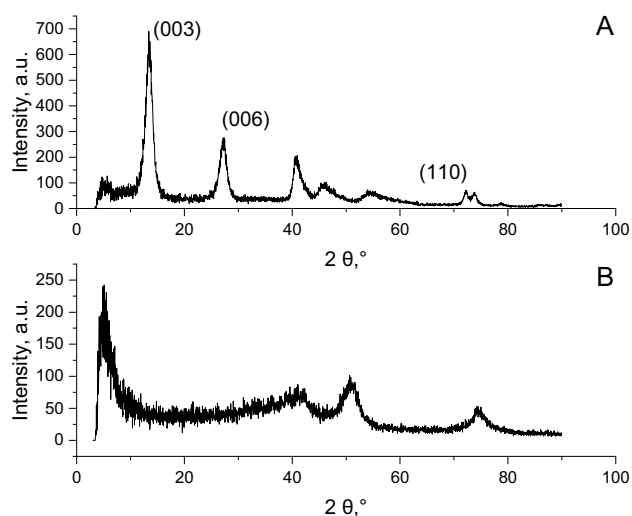


Fig. 1 XRD measurements of Hydrotalcite structure (A) and mixed oxide structure (B) obtained by calcination at 450 °C

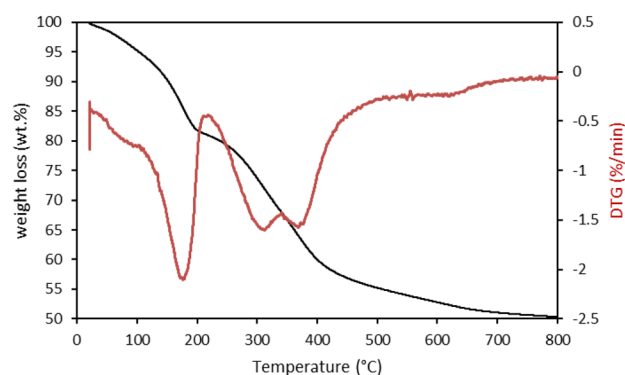


Fig. 2 Thermogravimetry measurement of hydrotalcite structure with DTG analysis

at 450 °C, the transition to an amorphous structure of the mixed oxide occurs, as evidenced by the XRD patterns in Fig. 1B.

During the transition from the layered crystalline structure of hydrotalcite to the structure of mixed oxide during

calcination, the interlayer space is removed. In Fig. 2, a thermogravimetric (TG) record of the weight loss during calcination is shown. The calcination process begins with the removal of physically adsorbed water from the structure at temperatures up to 100 °C. Subsequently, the removal of interlayer space occurs in two steps: first, dehydration, where water within the interlayer is eliminated; and second, decarbonation, where carbonate ions are removed from the interlayer. According to the TG record, the total weight loss is approximately 45 wt%, corresponding to the interlayer space. The transition to the structure of mixed oxide occurs at around 450 °C.

After the calcination of the prepared hydrotalcite and obtaining the structure of mixed oxides, additional properties were examined. One of them is the textural properties of the mixed oxide. In Fig. 3, an adsorption-desorption isotherm can be seen. From the shape of the hysteresis, it is evident that it is of type IV isotherm, which is characteristic of micro-mesoporous materials. Using T-plot measurements, the amount of micropores was determined, which was at zero value. The obtained structure of mixed oxides is therefore mesoporous, which is typical for mixed oxides. From the shape of the hysteresis loop, which is H3, the pore shape is wedge-shaped.

Figure 3, the pore size distribution of the measured sample can be seen. The measured sample exhibits a broad distribution with a peak around 12 nm. Since transesterification involves triglyceride molecules, which have a large diameter, increasing the pore size improves accessibility to the catalyst's active sites. The distribution also suggests the possible presence of macropores, which, however, cannot be precisely measured using BET analysis.

From the measurement of the textural properties, the specific surface area of the catalyst was determined to be 298 m²/g, as well as the pore volume, which is characteristic of mixed oxides (Table 1). The total basicity and acidity of the prepared mixed oxides were also measured. Since heterogeneous transesterification proceeds via a basic

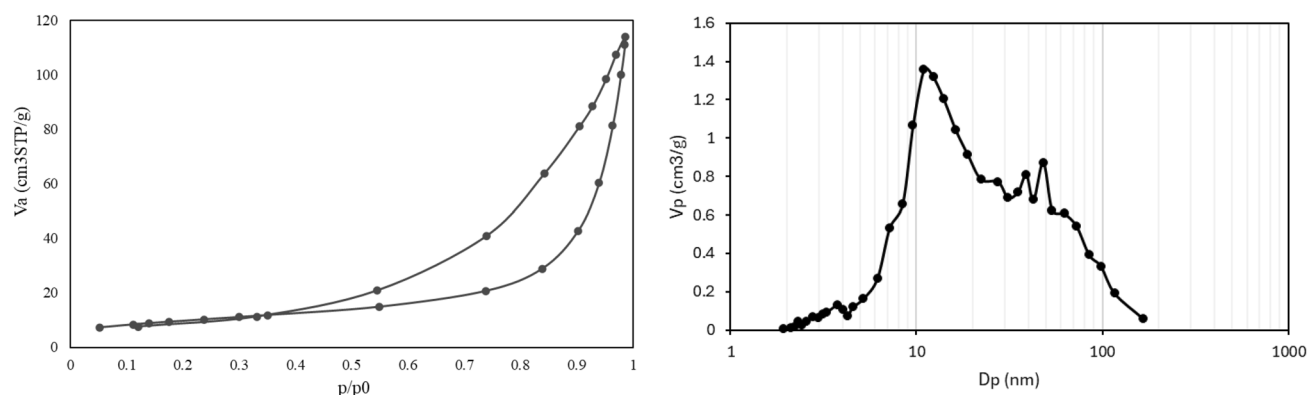


Fig. 3 Adsorption-desorption isotherm and pore distribution of mixed oxides calcinated at 450 °C

Table 1 Measured properties of mixed oxide obtained by calcination at 450 °C

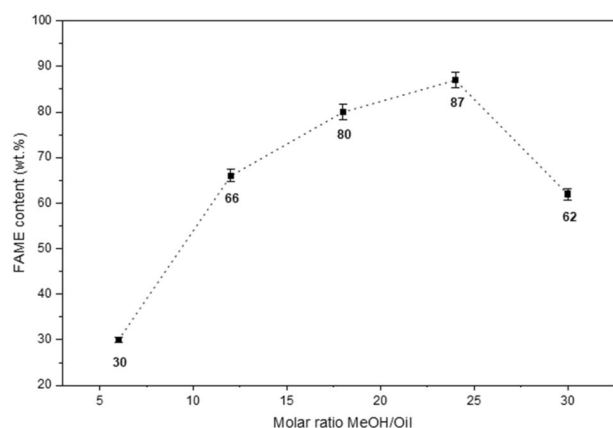
Property	Mg/Al/ Ni, molar ratio	SBET, (m ² /g)	V _p , (cm ³ /g)	Basicity, (mmol CO ₂ /g)	Acidity, (mmol/g)
Ni/MO	4/2.5/1	298	0.633	0.539	0.55

mechanism on mixed oxides, the number of basic sites is an important property. Conversely, the value of 0.55 mmol/g in the case of acidity is low, as the presence of acidic sites during transesterification is not required.

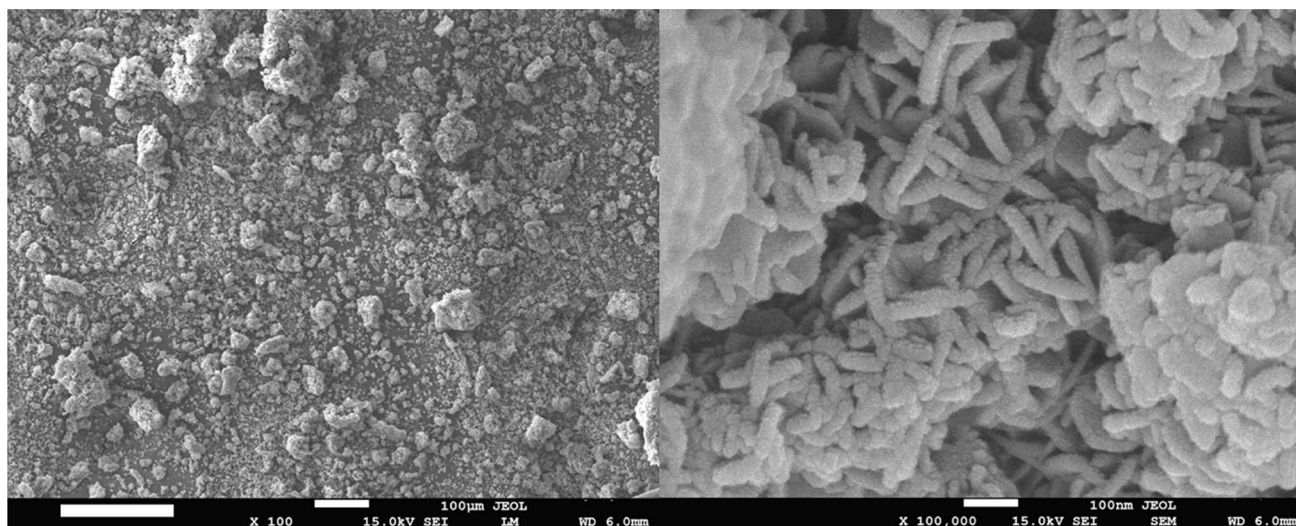
SEM images of the mixed oxide obtained after calcination at 450 °C can be seen in Fig. 4. The first image shows a 100x magnification of the structure. In the second image, the surface and structure of the Ni catalyst are magnified 100,000 times, allowing for a detailed view of the formed catalyst structures.

3.2 Molar Ratio

During the transesterification of vegetable oil, which primarily consists of triacylglycerols, the stoichiometry of the reaction requires 3 moles of methanol per mole of triacylglycerol. However, a methanol-to-oil molar ratio of 3:1 is insufficient to attain equilibrium in the system for the production of fatty acid methyl esters (FAME) due to the reversibility of the transesterification reaction. In homogeneous transesterification, an adequate methanol-to-oil molar ratio is 6:1, whereby this excess ensures the desired transesterification outcome. In heterogeneous transesterification, a larger amount of methanol and consequently a higher excess are necessary. Figure 5 illustrates the dependence of FAME content on the molar ratio, where the reaction temperature was maintained at 140 °C and the catalyst amount was set at 3 wt%.

**Fig. 5** Dependence of FAME content in biodiesel on the molar ratio of Methanol to oil with error bars

The observed dependence confirms the hypothesis that a molar ratio of Me/oil 6:1 is insufficient, as the amount of FAME obtained after 3 h is minimal. Increasing the molar ratio results in higher FAME yields, thereby increasing FAME content in the products. The larger quantity of methanol in heterogeneous transesterification can be explained by the necessity for increased contact between the oil phase, methanol, and the catalyst. Given that the reaction proceeds at 140 °C, where methanol exists in the gaseous phase, a sufficient excess is required to facilitate the reaction among the catalyst, oil, and methanol. As evidenced by the dependence, an excessively high excess of methanol relative to oil can also have negative effects; too much methanol may cause dilution of the oil, thereby reducing the reaction rate [29]. Although the optimal molar ratio of methanol to oil was identified as 24:1, the difference in FAME content between this ratio and 18:1 was only approximately 7%. From a technological and economic perspective, using the

**Fig. 4** SEM of mixed oxides obtained by calcination at 450 °C

lowest possible amount of methanol is preferable; therefore, subsequent experiments employed a molar ratio of methanol to oil of 18:1.

Yu et al. [20] also observed a similar trend, where increasing the molar ratio during transesterification led to a decrease in FAME yield. When the molar ratio of methanol to oil exceeded 25:1, only a slight increase in FAME yield was noted, and beyond a ratio of 35:1, a decline was observed. Other researchers have reported the same decrease in FAME yields with increasing methanol to oil ratios. Amani et al. [30], studying the transesterification of waste oil, observed a decrease in FAME yield when the methanol to oil ratio exceeded 20:1, which aligns with the trend observed in our results.

3.3 Temperature

The temperature of transesterification, as well as other reaction conditions, significantly influence the reaction outcome. The tested transesterification temperatures were set at four different levels. The amount of catalyst used was 3 wt%. Given that, during the optimization of the methanol/oil ratio, the most suitable ratio was found to be 18:1, the temperature tests were conducted using this methanol-to-oil ratio. With increasing reaction temperature, the transesterification proceeds faster, and therefore, the FAME yields and FAME content in the product are higher. Higher temperatures promote the attainment of equilibrium in the system and shift the equilibrium towards the formation of fatty acid methyl ester products.

As shown in Fig. 6, the maximum FAME content was achieved at a reaction temperature of 140 °C under the given conditions. Increasing the temperature by an additional 20 °C did not result in a rise in FAME content. Yu and colleagues [20] also investigated the optimal conditions for transesterification. However, in their case, it involved a mixture of oxides with calcium and cerium on pistachio oil. With the use of 9 wt% catalyst, they observed that due to the endothermic nature of transesterification, an increase in temperature led to an increase in FAME yield. However, they noted a decrease in yield when the temperature was raised from 107 °C to 122 °C. They attributed this decline to methanol evaporation and deteriorated contact between the reactant phases.

3.4 Pressure before Reaction

During transesterification using a heterogeneous catalyst, it is necessary to ensure adequate contact between all phases to facilitate the diffusion of reactants to the active sites of the catalyst. In heterogeneous transesterification at a temperature of 140 °C and atmospheric pressure, methanol is in

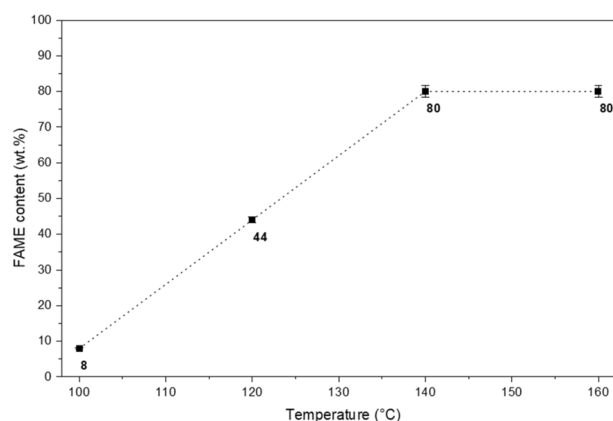


Fig. 6 Dependence of FAME content on Temperature during reaction with error bars

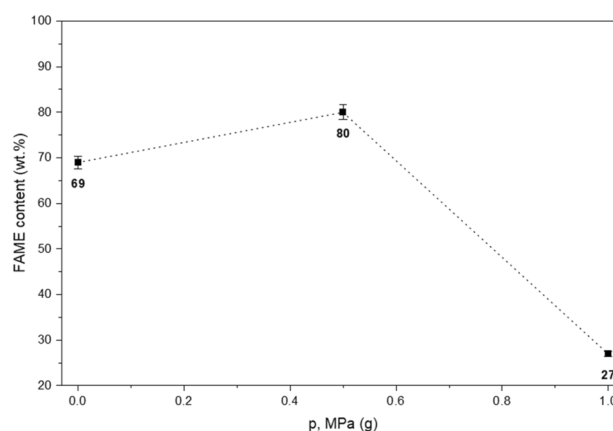


Fig. 7 Dependence of FAME content on pressure in the reaction before the reaction with error bars

the gaseous phase. Compressing it back into the liquid phase using nitrogen pressurization of the reactor proved to be effective for transesterification. For this reason, the required nitrogen pressure prior to transesterification was tested. The reaction conditions were based on already optimized parameters, with the methanol/oil ratio set at 18:1, catalyst amount at 3 wt%, and reaction temperature at 140 °C for 3 h. Figure 7 shows the dependence of FAME content on the nitrogen pressure in the reactor before transesterification. The most suitable pressure was found to be 0.5 MPa (g). With increasing pressure, the FAME content rapidly decreased.

3.5 Catalyst Amount

The amount of catalyst, both in heterogeneous and homogeneous transesterification, influences the course of the transesterification process as well as its outcome. In heterogeneous transesterification, a higher amount of catalyst naturally provides more active sites where transesterification occurs. With increased catalyst quantity, better contact

between the oil phase, methanol, and the catalyst is also expected, which can lead to higher yields in the reaction. However, this is limited by mixing, as sufficient agitation is necessary to ensure proper dispersion of the catalyst within the reaction mixture. Figure 8 illustrates the dependence of FAME content in biofuel on the amount of Ni/MO catalyst in the reaction. The tested catalyst amounts were used under already optimized conditions, with a methanol/oil ratio of 18:1 and a reaction temperature of 140 °C and pressure 0.5 MPa(g).

As is evident from the dependence, which is also confirmed by the hypothesis, increasing the amount of catalyst leads to an increase in FAME content in the product. However, as can be seen, the FAME content rises significantly up to a catalyst amount of 2.5%. There is no difference in FAME content between the levels of 2.5% and 3% and increasing the amount to 4% results in a similarly negligible increase. Ngamcharussrivichai and colleagues [31] studied the effect of conditions on palm oil transesterification. During their testing at 60 °C with a methanol-to-oil ratio of 30, they observed the same trend of increasing FAME content with the amount of catalyst. Beyond a catalyst amount higher than 3%, the increase in FAME content was negligible, and at 10%, the FAME yield did not differ from that at 8%. Yu and colleagues [20] observed a similar trend where increasing the catalyst amount led to higher FAME content in the sample; however, once the catalyst amount reached 6%, the increase plateaued, and further increases even resulted in a decrease in FAME yield. The authors attribute this trend to insufficient diffusion of methanol and oil into the catalyst. Amani and colleagues [30] also describe this decline, noting that during the transesterification of waste oil with catalyst amounts exceeding 3%, the FAME content in the products decreases. Gao and colleagues [11] also observed the effect of catalyst amount on FAME yield. In their reaction, they used a mixed oxide with potassium addition, with a reaction time of 3 h and a molar ratio of 12:1. From their observations, the same trend as in Fig. 8 is evident. When the catalyst amount exceeds 4%, the increase in FAME content is minimal; in their case, it even slightly decreases. They attribute this decline to the theory that the catalyst only affects the reaction rate but does not influence the equilibrium. This confirms that 3% catalyst and 3 h of reaction are sufficient conditions to reach equilibrium, and higher catalyst amounts do not shift the equilibrium towards higher FAME yields.

For practical use in heterogeneous transesterification processes, it is more advantageous to use the lowest possible amount of catalyst to achieve the desired results. This is both from an economic perspective and in terms of catalyst maintenance. Since the FAME content at 2.5% and 3% was the same, these amounts were tested further in a 7-hour

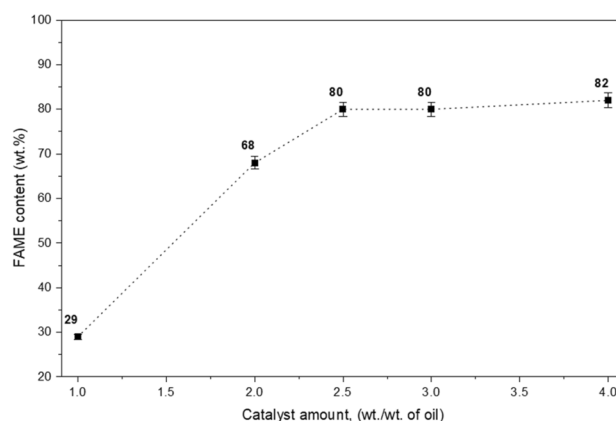


Fig. 8 Dependence of FAME content on catalyst amount with error bars

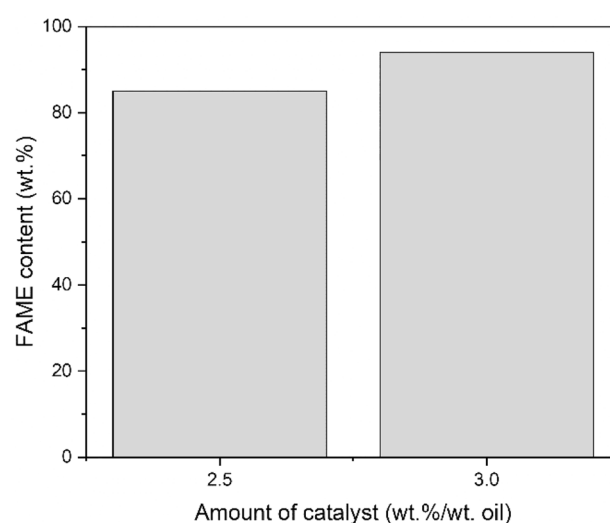


Fig. 9 Comparison of different amounts of catalyst in 7-hour reaction

reaction (Fig. 9). For the testing reaction, optimised conditions were used (140 °C, 18/1 molar ratio of MeOH/Oil, 0.5 MPa(g) in the reactor). Compared to 2.5% catalyst, 3% catalyst during 7 h of transesterification achieved a significantly higher FAME content. In the case of 2.5%, the FAME content was nearly 10% lower.

3.6 Reaction Time

The reaction time of three hours proved to be ideal for evaluating catalyst activity as well as testing individual conditions during transesterification. The influence of the reaction time on catalyst activity and the increase in FAME content is recorded in Fig. 10. The individual reactions for 1 h, 2 h, 3 h, 5 h, and 7 h were taken. For testing, already optimized conditions were used: catalyst amount of 3 wt%, methanol-to-oil ratio of 18:1, temperature 140 °C, and initial pressure of 0.5 MPa. As seen from the dependency, which also

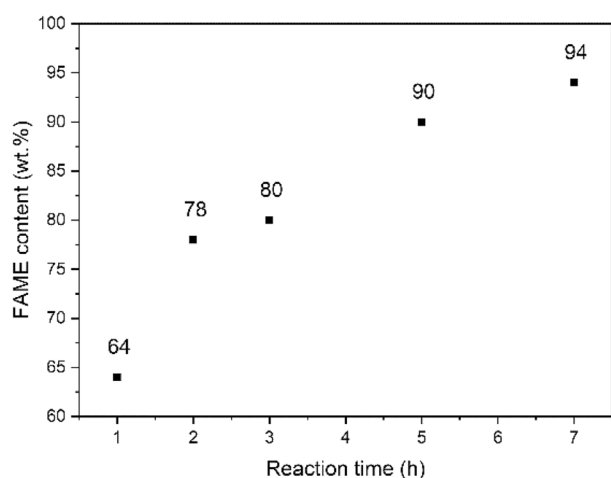


Fig. 10 Dependence of FAME content on reaction time in five different reactions

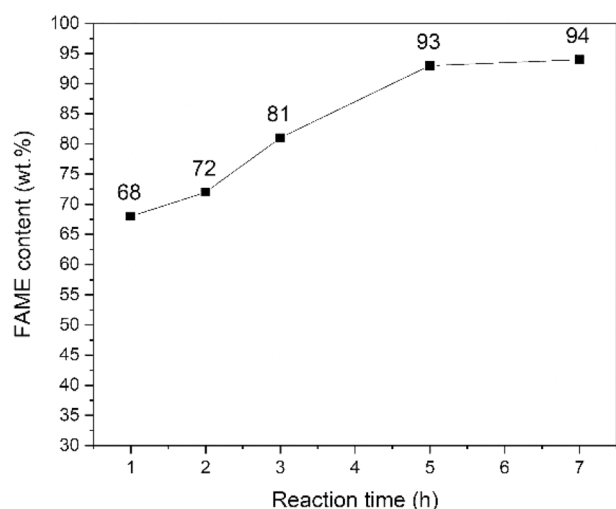


Fig. 11 Dependence of FAME content on reaction time in one reaction, with a sample taken during the reaction

confirms the assumption, a longer reaction time increases the FAME content in the samples.

Despite concluding individual reactions at the times shown on the dependency, it is possible to observe the attainment of a certain equilibrium after three hours. Although a longer reaction time will increase the FAME content in the biodiesel samples, the growth beyond three hours is minimal compared to the first three hours. From this perspective, it is not possible to say whether it is economically worthwhile to perform heterogeneous transesterification for 7 h, given that the difference between a six-hour and a seven-hour reaction is only about 94% FAME content.

The influence of reaction time on FAME content during the reaction was also tested in a single complex reaction, during which samples were taken at the same intervals as in the previous reactions: at 1 h, 2 h, 3 h, 5 h, and 7 h of

Table 2 FAME content in different steps of two-step transesterification

1. Step		2. Step	
Time (h)	FAME content (wt%)	Time (h)	FAME content (wt%)
3	79.4	2	89.2
3	81.5	3	94.7
2	71.5	2	90.5
7	94.0	-	-

the reaction. A small amount of sample was taken to avoid disturbing the equilibrium, but it was still possible to analyze the emerging FAME content during the reaction. From the course, it can be observed that initially, the Ni/MO mixture oxide is significantly active in transesterification with FAME content after only an hour, at a value of more than 68 wt%. However, after 3 h, the trend changes, with the sample containing an increase slowly (Fig. 11). After the third hour, only a slight increase in FAME content occurs. The final FAME content after 7 h is reaching 94%.

3.7 Two-Step Transesterification

From the course of transesterification with catalysts Ni/MO, it is evident that after three hours of reaction, a point is reached where the increase in FAME content is minimal, and even after seven hours of reaction, the FAME content does not meet the standard. For this reason, it was necessary to test a multi-stage transesterification process to further refine the oil to the desired value. After the first step of transesterification, i.e., 3 h under optimized conditions with the catalyst Ni/MO, an average FAME content of approximately 80 wt% was repeatedly achieved. Unreacted oil, corresponding to approximately 20 wt%, needed to be further reacted to reach at least the standard-compliant amount. For the transesterification, fresh catalyst (Ni/MO), calcined before the second stage of transesterification in an amount of 3 wt% relative to the unreacted oil, was used together with methanol in a ratio of 18:1 relative to the unreacted oil (20 wt%). Table 2 records the FAME content achieved after each step of transesterification. Various combinations of different times for the first and second step of transesterification were tested.

In the first experiment of the two-stage transesterification, a sample of *Camelina sativa* oil methyl esters after the first step, conducted for three hours with a content of 79.4 wt%, was re-reacted with fresh catalyst for 2 h, resulting in a FAME content of 89.2 wt%. The two-hour transesterification did not reach the desired value, so the reaction time of the second stage was increased by one hour in next experiment. In this case, the FAME content reached 94.7 wt%, which is comparable to single-stage transesterification with the same catalyst but with a reaction time of 7 h, whereas the total time for the two-stage transesterification was 6 h

(3+3 h). From a technological perspective, a shorter time to achieve the same FAME content is advantageous. However, considering the total amount of catalyst used, a higher amount of catalyst—about 15% more—is required for two-step transesterification compared to single-step. Regarding the total volume of methanol used for the two-step process relative to the single-step process, the methanol consumption does not differ. The volume of unreacted methanol from the first step, obtained after its evaporation, was higher than the needed amount of methanol (ratio 18:1) for the second step. Consequently, the volume of methanol required for both reaction steps did not increase. In the third attempt of the two-step transesterification, the duration of the first step was reduced to 2 h, and the second step was also adjusted to 2 h. The resulting FAME content showed that fresh catalyst amounting to 3 wt% relative to the unreacted oil from the first step (28.5 wt%)—which corresponds to approximately 0.4 g of catalyst per 71 g of mixture (unreacted oil + methyl esters)—is sufficiently active to nearly increase the FAME content by 20 wt%.

In all cases of the two-step transesterification, fresh methanol was used in both the first and second steps, dried with molecular sieves to ensure it contained no moisture. During transesterification on solid catalysts, which have both acidic and basic active sites, simultaneous transesterification and esterification can occur [32]. Since mixed oxides contain not only basic but also acidic sites, and the camelina sativa oil, has free fatty acids, it is assumed that simultaneous transesterification and esterification take place. The secondary product of esterification on acidic sites is water. To compare the yield of two-step transesterification in the case of direct use of distilled methanol without drying, a repeat of the two-step transesterification was performed under the same conditions but with unrefined (not dried) methanol (Table 3).

The FAME content results after the second step of transesterification with undried methanol directly used from the first step are lower under the same conditions. The presence of water formed in the first step and carried into the second step via methanol negatively affected the reaction outcome. Atadashi I.M. and colleagues [33] described the impact of water and moisture on the transesterification process. Several authors agree that in the case of transesterification on solid catalysts, moisture is not a limiting factor unlike in homogeneous systems, but in some cases, it can influence the course of transesterification [34, 35]. According to Yan S. and colleagues [36], the presence of a small amount of water does not improve or worsen the catalyst activity; however, a longer reaction time is needed to achieve the same FAME content during the reaction.

Although the results of the two-step transesterification were comparable to the one-step process with shorter

Table 3 FAME content in two-step transesterification

1. Step		2. Step	
Time (h)	FAME content (wt%)	Time (h)	FAME content (wt%)
3	79.5	3	91.6

Table 4 FAME content in two-step transesterification in compare with one-step

1. Step		2. Step	
Time (h)	FAME content (wt%)	Time (h)	FAME content (wt%)
3	79.9	3	97.8
7	94.0	—	—

reaction times, the results after the second step in neither case reached the minimum required FAME content according to the European standard. This may be due to the fact that the small amount of catalyst relative to the raw material (0.4 g of catalyst per 71 g of reactant) as well as the small amount of methanol (on average 12 ml of methanol per 80 ml of reaction mixture of unreacted oil and methyl esters from the first step) used in the second step for further reaction are limiting factors. Insufficient stirring and methanol at 140 °C in the gas phase, and thus inadequate contact among the oil, catalyst, and methanol phases, could have caused incomplete conversion to methyl ester products.

To achieve the minimum FAME content in the sample after transesterification and to overcome the limitation caused by insufficient contact of the small amount of catalyst and methanol, a two-step transesterification was tested with different ratios.

The first step of transesterification was carried out under optimized conditions. In the second step, the product from the first step was reacted with methanol in excess, with a ratio of 18:1, relative to the entire reaction mixture from the first step (i.e., unreacted oil + methyl esters), and not just the unreacted oil. In this case, an average amount of 49 ml of dried methanol was used per 61 g of oil. The amount of catalyst was also proportionally maintained at 3 wt% relative to the product from the first stage. Table 4 records the results of FAME content after each transesterification step. As can be seen from the table, after the second transesterification stage, the FAME content exceeded 96.5%, which is the standardized limit value.

Compared to a 7-hour reaction with the same catalyst, the two-stage transesterification with a total duration of 6 h achieved a higher FAME content. Regarding the used methanol, both the volume and the amount did not increase with the use of the second step, as in the previous case. After the completion of the first step of transesterification, an average of 50 ml of unreacted methanol was recovered, which is still higher than the required volume for the reaction in the second step. In the case of the catalyst, nearly twice the amount was used. For a single-step reaction lasting seven hours, 2.4 g of catalyst was used per 80 g of oil, whereas for

the two-step process, the total used on average was 4.1 g. The shortened reaction time, higher yield, and the same methanol consumption all favor the two-step transesterification process.

3.8 Catalyst Regeneration

Regeneration of the catalyst and its reuse in transesterification are considered advantages of heterogeneous transesterification. The catalyst yield after transesterification is nearly 100%; losses may occur due to partial leaching of metals as well as losses during separation. The used mixed oxide after transesterification is separated from the prepared biodiesel by decantation. Its regeneration involves washing the catalyst with acetone, and before the transesterification itself, it is calcined. The transesterification for regeneration testing was carried out under optimized conditions (Table 5).

The FAME content after 3 h of transesterification using Ni/Mo mixed oxide was 80%. This FAME content value was repeatedly achieved with the same catalyst under the same conditions. The Ni/Mo catalyst after transesterification was separated from the transesterification products, regenerated with acetone, and calcined overnight prior to the reaction. After the first regeneration of the catalyst, the activity of the mixed oxide in transesterification with camelina sativa oil significantly decreased, with the FAME content after 3 h with the regenerated catalyst dropping to 55.1%. After the second regeneration, the activity decreased even further, with the FAME content after 3 h using the twice-regenerated catalyst declining to 22.3%.

4 Conclusion

Heterogeneous transesterification, which is commonly used in biodiesel production, brings several disadvantages that heterogenous transesterification could potentially resolve. Additionally, the use of edible oils is a drawback in biodiesel manufacturing. Using heterogeneous transesterification on mixed oxides with unconventional oils such as camelina sativa can eliminate both of these issues. In this work, optimal conditions for transesterification were tested on mixed oxides prepared from hydrotalcite by calcination, doped with nickel into its structure. It was found that an optimal temperature for transesterification is 140 °C. Under these conditions, with a catalyst content of 3 wt% and a methanol-to-oil ratio of 18:1, the results were optimal. By pressurizing the reactor to 0.5 MPa(g) before the reaction under these optimal conditions, more than 94% FAME yield was achieved after 7 h. However, the product did not meet the European standard in FAME content. Therefore, a two-step transesterification process was implemented, where the

Table 5 FAME content after two regenerations

	First reaction	First regeneration	Second regeneration
FAME content (wt%)	80.0	55.1	22.3

reaction was stopped after three hours, glycerol and methanol were separated, and unreacted oil with biodiesel was sent to a second step. Here, the reaction continued for an additional 3 h, enabling the achievement of over 97 wt% FAME content, which complies with European standards.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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