OPTIMIZATION OF QUICKLIME-CATALYZED BIODIESEL PRODUCTION FROM HEMP (*CANNABIS SATIVA* L.) SEED OIL

Biljana S. Đorđević, Milan D. Kostić, Marija R. Miladinović, Olivera S. Stamenković

Faculty of Technology, University of Niš, Leskovac, Serbia

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This study focuses on the development of a sustainable biodiesel production process using non-edible hemp seed oil (HSO) and an environmentally friendly, low-cost quicklime catalyst. HSO was recovered via cold-pressing, with major fatty acids identified as linoleic acid (55.70%), linolenic acid (15.59%) and oleic acid (13.72%). Due to the slightly elevated free fatty acids (FFAs) content in HSO, a two-stage process was employed: esterification of FFAs in HSO catalyzed by sulfuric acid, followed by methanolysis of the esterified HSO using quicklime as a catalyst. The methanolysis reaction was optimized using a 3^3 factorial design with three central points, combined with response surface methodology (RSM). The optimal reaction conditions for achieving maximum fatty acid methyl esters (FAME) content were determined to be: methanol-to-HSO molar ratio of 6:1, catalyst concentration of 6.8%, and reaction time of 45 min. Additionally, the produced biodiesel meets the EN 14214 biodiesel quality standard specifications, with the exception of the iodine value. Overall, the methanolysis of non-edible HSO using low-cost quicklime presents a promising, sustainable approach for biodiesel production.

Keywords: Biodiesel; hemp seed oil; optimization; methanolysis; quicklime

Introduction

The escalating global demand for energy in the 21st century, projected to double by 2050, is inevitably driving the depletion of fossil fuel reserves [1]. The impending shortage and the environmental challenges associated with fossil fuel consumption have spurred the urgent need for sustainable alternatives. As a result, renewable energy sources such as biodiesel, a mixture of fatty acid alkyl esters, have emerged as a viable solution. Biodiesel is produced through the catalyzed transesterification of triacylglycerols (TAGs) from oily feedstock with alcohol, usually methanol. This biofuel has the potential to serve as a clean, renewable, non-toxic, and biodegradable energy source, offering an environmentally friendly alternative to diesel derived from fossil fuels [2,3].

Over the past years, various biodiesel production processes have been developed involving transesterification catalyzed by a range of homogeneous [4] and heterogeneous catalysts [4,5] including nanocatalysts [6] or enzymes [4,7]. These processes have been further enhanced by the use of cosolvents [8], ultrasound [9], or microwaves [10]. Biodiesel has been produced from a variety of feedstocks, including edible oils [11], non-edible oils [12], and waste oily feedstocks [13]. In an effort to mitigate the use of edible oils, which plays a crucial role in human nutrition, the scientific community has shifted its focus towards utilizing non-edible and waste oils for biodiesel production.

Cannabis sativa L., commonly known as hemp, is an annual, dioecious plant belonging to the Cannabinaceae family, native to central Eurasia [14,15]. Hemp seeds, recognized as valuable feedstocks for various products, including fuels [16], have been gaining renewed attention with the potential for increased cultivation [17]. In addition to proteins (20–35%) and carbohydrates (20–30%), hemp seeds are rich in fatty oil (25–35%) [18].

The high oil content and the adaptability of hemp to diverse agro-ecological conditions make it an attractive source for biodiesel production. According to the techno-economic analysis of biodiesel and bioethanol co-production from industrial hemp conducted by Viswanathan *et al*. [19], between 150 and 750 liters of biodiesel per hectare of cultivated land can be produced from hemp, depending on the lipid content in the biomass, which ranges from 2% to 10%. Furthermore, it has been estimated that 51% more biodiesel can be produced from a hectare of hemp compared to a hectare of soybean. Biodiesel production from hemp seed oil (HSO) has been extensively studied using homogeneous catalysts [20-28], whereas investigations employing heterogeneous catalysts remain relatively scarce [1,29,30]. Abbasi

*Author address: Biljana Đorđević, University of Niš, Faculty of Technology, Leskovac
Bulevar Oslobođenja 124, 16000 Leskovac, Serbia
E-mail address: djbiljana89@gmail.com
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et al. [1] investigated the use of green WO_3 nanoparticle synthesized via a biological method employing *C. sativa* leaf extracts for biodiesel production from HSO. Bi-functional catalysts comprising alkaline earth oxides loaded with copper oxide, prepared through the chemisorption-hydrolysis method, were used in the transesterification-selective hydrogenation process for biodiesel production from HSO [29,30]. However, these studies primarily focus on oils with low free fatty acids (FFAs) content, and the reported catalyst required complex preparation methods that could limit their scalability. To address this issue, this study proposes the use of quicklime as a catalyst, which is an inexpensive and naturally abundant material, activated through a simple thermal treatment. Furthermore, optimizing reaction conditions has emerged as a pivotal strategy in process development. Several studies on biodiesel production from HSO in the presence of homogeneous catalysts have focused on this aspect [26-28]. Notably, Abbasi *et al.* [1] optimized the heterogeneous-catalyzed methanolysis of HSO using central composite design and response surface methodology (RSM).

This study focuses on the quicklime-catalyzed methanolysis of HSO obtained through cold pressing. The fatty acid methyl esters (FAMEs) production was achieved through a two-stage process: esterification of FFAs in HSO using sulfuric acid as a catalyst, followed by methanolysis of the esterified HSO in the presence of quicklime. To evaluate the feasibility of HSO methanolysis over quicklime, this research aimed to optimize the methanolysis reaction conditions. The optimization process was conducted using 3^3 factorial design with three central points, combined with RSM. Finally, the properties of the resulting HSO biodiesel were analyzed in accordance with the biodiesel quality standard.

Material and methods

Materials

Hemp seeds and quicklime were procured from a local pet shop and market, respectively. Methanol (99.5%) and Na_2SO_4 were supplied by Zorka-Pharma (Serbia). Concentrated H_2SO_4 was obtained from Lach-Near (Czech Republic), while Na_2CO_3 was purchased from Sigma Aldrich (St. Louis, USA). HPLC grade *n*-hexane and methanol were supplied by Promochem LGC (Germany) and HPLC grade 2-propanol was procured from Carlo Erba (Italy).

Extraction and characterization of HSO

HSO was extracted using the cold pressing method. The screw oil press (Komet, Germany) with 10 mm nozzles was utilized for seeds pressing. The extracted oil was vacuum-filtered using a Buchner funnel to remove residual hemp seed particles. Subsequently, the physicochemical properties of the obtained oil were determined. Acid, iodine, and saponification values were determined in accordance with the AOCS official methods [31]. The density and viscosity of the oil were measured at 20 °C using a pycnometer and a rotational viscometer (Visco Basic Plus v. 0.8, Fungilab S.A., Barcelona, Spain). The fatty acid composition of HSO was determined by gas chromatography following the methylation of the oil as prescribed by SRPS EN ISO 12966-2:2011 and SRPS EN ISO 5508:2009 standards.

Biodiesel production

Due to the acid value of HSO (2.98 mg KOH/g) exceeding the acceptable limit (1 mg KOH/g) for direct base-catalyzed methanolysis, a two-stage process was used for biodiesel production. In the first stage, the FFAs in HSO were esterified with methanol in the presence of sulfuric acid as a catalyst. The reaction was conducted under the following conditions: a methanol-to-HSO molar ratio of 8.5:1, catalyst concentration of 2%, and reaction temperature of 45 °C, as described by Kostić *et al.* [32]. After the completion of the reaction (60 min), the oil-ester phase was gravitationally separated from the alcohol phase using a separatory funnel. The separated ester-oil phase was washed with distilled water heated to 50 °C to remove residual H_2SO_4, followed by drying with anhydrous Na_2SO_4 and filtration. As a result, the acid value of esterified HSO was reduced to 0.58 mg KOH/g oil, making it suitable for use in the base-catalyzed methanolysis reaction.

In the second stage, the esterified HSO underwent methanolysis catalyzed by quicklime, which had been pre-treated by crushing to a particle size of 0.5 mm and thermally activated at 550 °C for 4 h. The characteristics of the raw and calcined quicklime can be found elsewhere [33]. Methanolysis was performed in a three-neck round-bottom glass flask (250 mL) equipped with a reflux condenser and a magnetic stirrer, following the procedure outlined by Kostić *et al*. [32]. The methanolysis reaction was conducted under varying conditions, including catalyst concentrations of 3, 5, and 7%, based on the oil mass; methanol-to-HSO molar ratios of 6:1, 9:1 and 12:1; and reaction times of 20, 40, and 60 min, while maintaining a constant temperature of 60 °C. The composition of the ester-oil phase was analyzed using high-performance liquid chromatography (HPLC), as detailed by Stamenković *et al.* [34].

Statistical modelling and optimization of esterified HSO methanolysis

To optimize the methanolysis of esterified HSO, a factorial design 3^3 with three central points in combination with RSM was employed. The experiments were conducted in random order to minimize systematic errors. The significance of the individual factors, their interactions and the overall applicability of the model for fitting the experimental data were assessed using analysis of variance (ANOVA) with Design-Expert 7.0.0 software (Stat-Ease Inc., Minneapolis, MN). The optimal reaction conditions for maximizing FAME content were determined by solving a quadratic model equation (Eq. 1):

$$y = b_o + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + \\ + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \quad (1)$$

where A is the catalyst concentration (%), B is the methanol-to-HSO molar ratio (mol/mol), C is the reaction time (min), y is the FAME content (%), b_0, b_i, b_{ii} and b_{ij} (i, j = 1, 2, 3, and $i \leq j$) are regression coefficients.

FAME purification and characterization

The reaction mixture obtained from the methanolysis of esterified HSO under optimal reaction conditions was filtered to remove the spent catalyst. Subsequently, the liquid phase was gravitationally separated into the alcoholic phase (containing methanol and glycerol) and the ester phase (crude biodiesel). The crude biodiesel was then purified following the procedure described by Alba-Rubio *et al*. [35]. The physicochemical properties of the purified biodiesel were determined using standard methods, including: density (EN ISO 3675:1988), viscosity (EN ISO 3104:2003), acid number (EN 14104:2003), iodine number (EN 14111:2003), water content (EN ISO 12937:2000), as well as the FAME content (EN 14103:2003), and monoacylglycerol (MAG), diacylglycerol (DAG) and TAG contents (EN 14105:2003).

Results and discussion

Physicochemical properties and fatty acid composition of HSO

The yield, physicochemical properties, and fatty acid composition of the obtained HSO are presented in Table 1, along with a review of the previously reported studies on HSO obtained by various extraction techniques. The oil yield obtained by cold pressing of hemp seeds was determined to be 25.45±0.07 g/100 g, which agrees with previously reported studies [24,36-38]. Notably, Muangrat *et al*. [39] reported a significantly lower yield for HSO obtained through ultrasound-assisted and low-pressure supercritical CO_2 extraction. The oil yield from cold pressing of hemp seeds was higher than that obtained from white mustard seeds (20.64±0.18 g/100 g, corresponding to 64.3% of the maximum oil yield) [40].

Table 1. Yield, physicochemical properties, and fatty acid composition of HSO

Properties	HSO												
Oil yield, g/100 g	25.45±0.07	26.90±0.50 – 31.50±0.28						24.50±2.00	26.89				13.46 – 15.93
Density, kg/m^3 (20 °C)	0.918	0.918±0.003 –0.927±0.003[a]		0.925									
Viscosity, mPas (20 °C)	96.8			97									
Water content, %	1.89												
Acid value, mg KOH/g oil	2.98		0.10	1.30				2.05	0.78	1.78±0.18		0.89±0.02 – 4.58±0.03	24.84±1.19 – 45.09±0.07
Iodine number, g I_2/100 g oil	152.5	154±1.7 – 165±1.6	164.0	146.0				149.8	153.2				102.2±1.5 –114.1±1.6
Saponification value, mg KOH/g oil	185.8	184±1.7 – 190±1.9		187.0				192.9					187.8±4.2 – 222.7±0.27
Fatty acids, %													
C16:0	6.78	5.75±0.16 – 8.27±0.18	6.64	6.63	7.31	7.41	6.92	7.00	7.15±0.42	6.25	7.68	6.95	4.95±0.12 – 7.12±0.05	6.72±0.04 –7.58±0.04
C16:1														0.11±0.02 – 0.13±0.04
C18:0	2.89	2.19±0.15 – 2.79±0.13	2.76	3.18					2.73±0.21	2.96	2.73	2.68		2.72±0.01 – 2.99±0.05
C18:1	13.72	10.17±0.17 – 14.03±0.14	10.12	13.18	13.00	13.27	13.17	13.5	12.75±1.10	13.99	14.19	12.31	6.89±0.12 – 20.5±0.29	15.73±0.02 – 16.65±0.09
C18:2	55.70	56.50 ± 1.00 –60.50 ± 0.80	54.31	55.96	58.25	57.94	58.19	55.2	56.08±3.05	56.33	55.84	56.16	38.50±0.47 – 52.20±2.36	57.08-0.02 – 57.63±0.23
C20:0									0.89±0.05	0.93	0.32	0.50		0.41±0.06 – 0.53±0.06
C20:1	3.09								0.26±0.02	3.47	0.37	0.24		
C18:3	15.59	16.85±0.45 – 20.00±0.39	23.76	19.34	19.44	19.28	19.45	20.50	17.92±1.18	15.63	17.46	20.07	13.31±0.14 – 21.67±0.26	14.78±0.19 – 15.31±0.07
C20:2									1.03±0.32					0.07±0.01 – 0.11±0.00
C22:0									0.20±0.06	0.47			0.16±0.00 – 0.29±0.00	0.36±0.00 – 0.41±0.00
Saturated fatty acids	9.67	7.94–11.06	9.40	9.81	7.31	7.41	6.92	10.50	10.97±0.47	10.61	11.23	9.68	6.89 – 9.47	10.06±0.06 – 11.03±0.08
Monounsaturated fatty acids	16.81	10.17–14.03	10.20	13.18	13.00	13.27	13.17	13.5	13.01±1.10	17.46	15.28	12.65	6.97–20.84	16.28±0.01 – 17.31±0.01
Polyunsaturated fatty acids	71.29	73.35–80.5	78.07	75.30	77.69	77.22	77.64	75.7	75.03±3.31	71.96	71.85	77.67	52.59–70.38	72.60±0.12 – 72.72±0.08
Extraction methods	Cold pressing	Cold pressing[b]	Refined oil	Cold pressing	Cold pressing	Soxlet	SC-CO_2	Cold pressing	Cold pressing	Cold pressing	Cold pressing	Cold pressing	Cold pressing[c]	Ultrasound and low-pressure SC-CO_2[d]
Reference	This work	[37]	[29]	[26]	[43]			[25]	[38]	[24]	[44]	[45]	[46]	[39]

[a]at 24 °C,
[b]Three hemp seed oils samples,
[c]Thirteen samples of different commercial cold pressed HSO,
[d]Under different extraction conditions.

Furthermore, it was comparable to the yield obtained from cold pressing of plum kernels (25.5 g/100 g, representing 71% of the best oil yield) [32] and black mustard seeds (22.35±1.34 g/100 g, corresponding to 71.9% of the best oil yield) [41]. The physicochemical properties of the obtained HSO closely align with the available literature data (Table 1), with the exception of the acid value. This difference can be attributed to the influence of factors such as plant cultivation and genetics variations [42], seed storage duration and conditions [32], as well as the extraction technique and parameters employed [39]. Unsaturated fatty acids predominate in HSO, comprising 85.01% of the total fatty acids. The major fatty acids include linoleic acid (55.70%), linolenic acid (15.59%) and oleic acid (13.72%) (Table 1), which is consistent with the high iodine value of this oil. Furthermore, the fatty acid composition of HSO is in agreement with the previously published data (Table 1).

Optimization of esterified HSO methanolysis

The methanolysis of esterified HSO was conducted under varying catalyst concentrations (factor *A*), methanol-to-HSO molar ratios (factor *B*), and reaction times (factor *C*), following a factorial design 3^3 with three central points. The experimental matrix, including both coded and actual factor values, along with the experimentally determined and predicted FAME content values, is presented in Table 2.

Table 2. Experimental matrix for factorial design 3^3 with three central points

Run no.	Coded values			Actual values			FAME content, %	
	Factor						Experimental	Predicted
	A	*B*	*C*	*A*, %	*B*, mol/mol	*C*, min		
1	-1	-1	-1	3	6:1	20	10.0	11.79
2	0	-1	-1	5	6:1	20	21.3	18.26
3	1	-1	-1	7	6:1	20	26.4	25.12
4	-1	0	-1	3	9:1	20	13.10	10.07
5	0	0	-1	5	9:1	20	12.60	16.47
6	1	0	-1	7	9:1	20	18.40	23.27
7	-1	1	-1	3	12:1	20	10.30	9.77
8	0	1	-1	5	12:1	20	14.80	16.11
9	1	1	-1	7	12:1	20	26.80	22.84
10	-1	-1	0	3	6:1	40	85.50	86.61
11	0	-1	0	5	6:1	40	90.40	90.06
12	1	-1	0	7	6:1	40	93.30	93.91
13	-1	0	0	3	9:1	40	86.10	85.99
14	0	0	0	5	9:1	40	89.90	89.38
15	1	0	0	7	9:1	40	93.80	93.16
16	-1	1	0	3	12:1	40	85.50	86.81
17	0	1	0	5	12:1	40	88.30	90.13
18	1	1	0	7	12:1	40	94.30	93.84
19	-1	-1	1	3	6:1	60	95.90	96.65
20	0	-1	1	5	6:1	60	96.90	97.09
21	1	-1	1	7	6:1	60	97.70	97.92
22	-1	0	1	3	9:1	60	98.10	97.15
23	0	0	1	5	9:1	60	98.10	97.51
24	1	0	1	7	9:1	60	98.40	98.28
25	-1	1	1	3	12:1	60	99.40	99.07
26	0	1	1	5	12:1	60	99.30	99.37
27	1	1	1	7	12:1	60	99.30	100.07
28	0	0	0	5	9:1	40	91.10	89.38
29	0	0	0	5	9:1	40	90.80	89.38
30	0	0	0	5	9:1	40	89.00	89.38

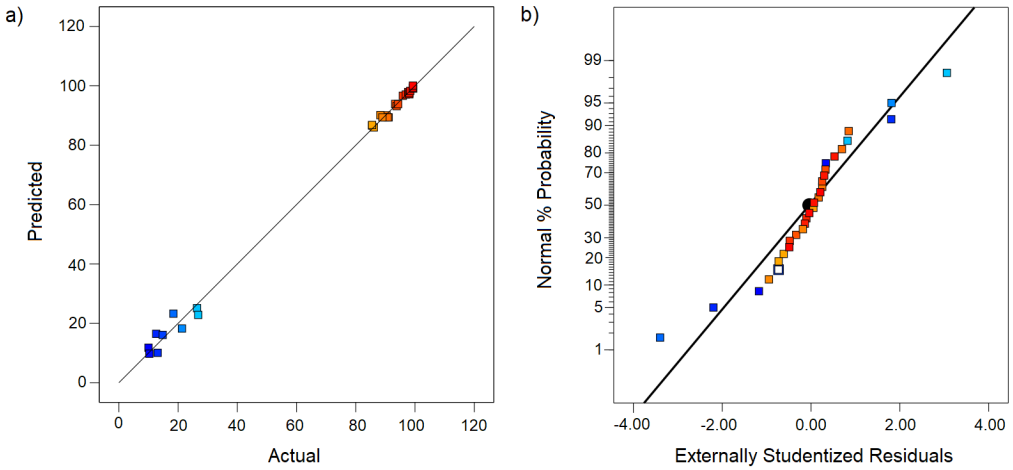

Figure 1. Graphical presentation of statistical results: Predicted *vs.* Actual FAME contents (a) and Probability plot (b)

The experimentally obtained values of FAME content (response) were correlated with the reaction conditions (factors) using a quadratic model (Eq. 1). The significance of the model was confirmed by a high *F*-value (874.56) and a low *p*-value (< 0.0001). The model demonstrated excellent fit, as indicated by high coefficient of determination (R^2 = 0.997), and the adjusted and predicted coefficients of determination (R^2_{adj} = 0.996; R^2_{pred} = 0.994), all of which are close to 1. Additionally, the lack of fit was not significant (*F* = 6.03, *p* = 0.082 > 0.05), further supporting the adequacy of the model in fitting the experimental data. A good agreement between the actual and predicted FAME content values was observed (Fig. 1a), as reflected in the low mean relative percent deviation (*MRPD*) value (±6.04%, based on 30 data points), indicating the reliability of the model. The validity of the model was also confirmed by the normal distribution of residuals (Fig. 1b).

The effects of process factors and their interactions on FAME content were evaluated using the ANOVA method. Based on the *F*- and *p*-values (Fig.2a), the catalyst concentration (*A*), reaction time (*C*), their interaction (*AC*), and the quadratic term of reaction time (C^2) were found to have a statistically significant influence on FAME content at the 95% confidence level. Among these factors, reaction time exhibited the most pronounced effect on FAME content (*F*-value = 6221), followed by catalyst concentration (*F*-value = 48.7). Both factors positively influenced FAME content, as indicated by the positive values of their linear regression coefficients (Fig. 2b). FAME content increased rapidly with the reaction time up to approximately 50 min (coded value 0.5), as evidenced by the steep slope of the reaction time curve in the perturbation plot (Fig. 3). However, with an extended reaction time, a noticeable decline in FAME content was observed. This phenomenon can be attributed to the reversible nature of the reaction, leading to a reduction in FAME content during prolonged reaction times [47]. A similar effect of reaction time on FAME content has been previously reported for the methanolysis of *Jatropha curcas* oil [48], gurgure oil [49], waste frying oil [50], and beef fat [47], all catalyzed by CaO-derived catalysts. Reaction time has also been recognized as a pivotal factor influencing FAME production in various processes. Abbasi *et al*. [1] reported that reaction time had the most pronounced effect on FAME formation in WO_3-catalyzed methanolysis of HSO, surpassing the influence of metanol-to-oil molar ratio, catalyst concentration and reaction temperature. Similarly, in the CaO-catalyzed methanolysis of waste frying oil, reaction time was identified as the most influential factor, exceeding the effects of methanol-to-oil molar ratio and catalyst concentration [51].

The second factor that significantly and positively influences FAME content is catalyst concentration (Fig. 2). Its effect is lower compared to reaction time through the whole investigated range (Fig. 3). The increase in FAME content with higher catalyst loading is attributed to the enhanced availability of catalytically active sites for the methanolysis reaction. Namely, as the catalyst concentration increases, more active sites become accessible for the interaction between TAG and methanol, thereby accelerating the reaction rate, and leading to a more efficient conversion of TAG into FAME [47]. The positive correlation between increased catalyst loading and enhanced FAME formation has been previously reported for rapeseed oil methanolysis over CaO-based catalysts [52,53].

On the other side, the effects of methanol-to-HSO ratio (*B*), its interaction with catalyst concentration (*AB*) and reaction time (*BC*), as well as the quadratic terms of catalyst concentration (A^2) and methanol-to-HSO ratio (B^2) were found to be statistically insignificant on FAME content (Fig. 2a). The negligible influence of methanol-to-HSO ratio is further supported by the nearly horizontal curve in the perturbation plot (Fig. 3). The observed insignificant effect of methanol-to-oil molar ratio can be attributed to several factors. Excess methanol can saturate catalyst particles, reducing the availability of active sites for TAG

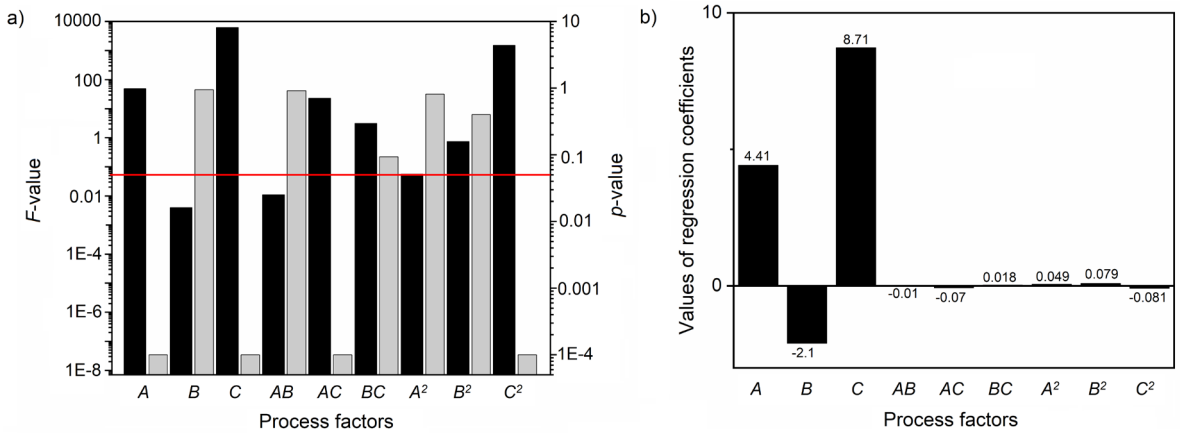

Figure 2. Pareto chart (*F*-values - black bars, *p*-values - grey bars) (a) and the values of regression coefficients (b)

molecules and limiting the effective reactant interactions [49]. Additionally, excess methanol increases the solubility of glycerol, a by-product of the reaction, which shifts the reaction equilibrium backward toward the reactants, reducing FAME yield [54]. Similarly, Abbasi *et al.* [1] reported an insignificant effect of the methanol-to-oil molar ratio on FAME content during HSO methanolysis catalyzed by WO_3 nanocatalyst. A statistically insignificant effect of the methanol-to-oil molar ratio on FAME content has also been observed for the methanolysis of refined palm oil [55,56], neem oil [57], waste used oil [54,58], sour cherry kernels oil [59], and blended waste cooking oil [60,61].

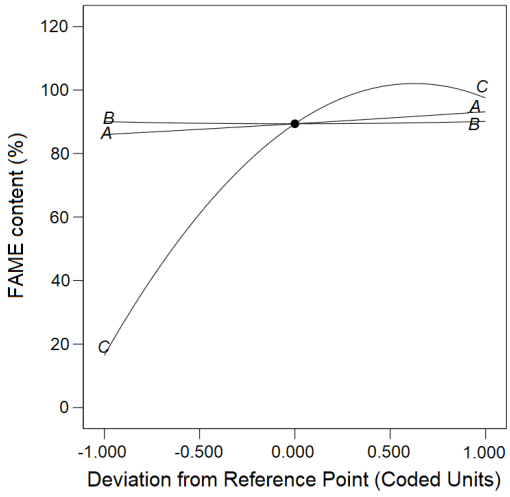

Figure 3. Perturbation plot

The influence of catalyst concentration and reaction time on FAME content at a methanol-to-HSO molar ratio of 6:1 is presented as a 3D plot in Fig. 4. The results indicate that FAME content increases slightly with rising catalyst concentration, with this effect being more pronounced at a reaction time of 20 min. Specifically, increasing the catalyst concentration from 3% to 7% at a reaction time of 20 min resulted in an increase in FAME content from 12% to 27%. At longer reaction times (beyond 50 min), the influence of catalyst concentration became negligible. Conversely, increasing the reaction time from 20 to 40 min at a catalyst concentration of 3% led to a substantial increase in FAME content, from 12% to 86%. Extending the reaction time to 50 min yielded a maximum FAME content of 100%. Subsequently, the FAME content plateaued and then slightly decreased. Prolonged reaction times could shift the reaction equilibrium toward the reactants, thereby promoting soap formation and consequently reducing FAME content [48,62-64].

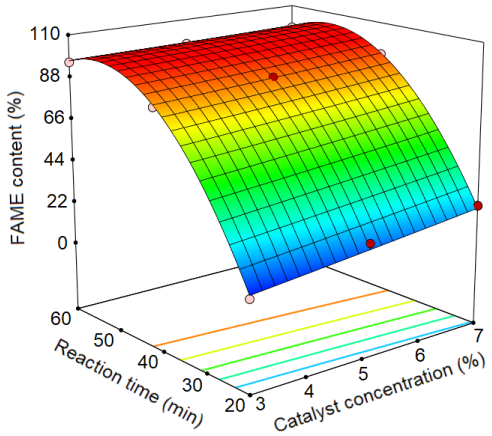

Figure 4. 3D surface plots of FAME content as a function of reaction time and catalyst concentration (at methanol-to-HSO molar ratio 6:1)

The process optimization was conducted with methanol-to-HSO molar ratio fixed at 6:1, as its effect on FAME content was negligible. This approach aimed to mitigate challenges in the downstream process associated with FAME separation and methanol recovery at higher methanol amounts [47]. Consequently, the optimal reaction conditions for achieving maximum FAME content were determined by solving the model equation (Eq. 1). The software suggested eight combinations of process factors, yielding FAME content from 99.8% to 103.1%. By selecting the shortest reaction time as an additional criterion, the optimal reaction conditions were identified as a catalyst concentration of 6.8% and a reaction time of 45 min. The close agreement between predicted (100.6%) and experimentally obtained (99.1%) FAME content under chosen optimal conditions confirms the reliability of the developed model. For comparison, the optimal reaction conditions for HSO methanolysis over WO_3 nanocatalyst were reported as a catalyst concentration of 2%, methanol-to-HSO molar ratio of 7:1, reaction temperature of 80 °C, and reaction time of 120 min, achieving a FAME content of 91% [1]. The determined optimal reaction conditions are similar to those reported for the methanolysis of white mustard seed oil catalyzed by quicklime (a methanol-to-oil molar ratio of 6.1:1, catalyst concentration of 9.8%, and reaction time of 50 min) [65].

Physicochemical properties of purified FAME obtained from HSO

The physicochemical properties of biodiesel obtained from HSO are presented in Table 3 and compared with literature data for biodiesel produced from HSO, as well as with the values specified by the EN 14214 biodiesel quality standard. The values of the physicochemical properties determined in the present study are consistent with those reported in previous studies on biodiesel derived from HSO. Furthermore, all the determined values fall within the limits set by the EN14214 quality standard,

Table 3. Properties of FAME obtained from HSO

Property	FAME obtained from HSO										EN 14214
Density (15 °C), kg/m^3	881	884	872	892	885	890	810	872	875	886±0.45	860-900
Viscosity (40 °C), mm^2/s	3.80	3.48	4.23	3.83	3.90	5.17	4.82	5.10	3.40	3.09±0.01	3.5-5.0
Acid value, mg KOH/g oil	0.31	0.25	0.01	0.20	0.25	0.45	0.41		0.44	0.20±0.01	0.50 max
Iodine number, g I$_2$/100 g oil	151		164		154			163	165		120 max
Water content, mg/kg	275				150		10				500 max
FAMEs, %	99.1				98.5						96.5 min
MAGs, %	0.5				0.5						0.8% max
DAGs, %	0.2				0.2						0.2% max
TAGs, %	0.2				0.1						0.2% max
Reference	This work	[70]	[30]	[20]	[26]	[25]	[21]	[23]	[27]	[22]	

except for the iodine number, which exceeds the specified limit. Some researchers argue that the restriction of iodine value specified in the EN14214 standard is unnecessary [66]. Moreover, the addition of natural antioxidants such as tocopherols, β-carotene, ascorbic acid, and flavonoids [67-69], as well as additives [70] can significantly improve the oxidation stability of biodiesel.

Conclusion

This study demonstrated the effective utilization of HSO as a feedstock for biodiesel production, with quicklime applied as a catalyst for the first time. Cold pressing yielded 25.45±0.07 g/100 g of HSO, comprising 85.01% unsaturated fatty acids. Due to the elevated acid value of HSO (2.98 mg KOH/g), a two-stage process was employed: acid-catalyzed esterification of FFAs, which reduced the acid value to 0.58 mg KOH/g, followed by quicklime-catalyzed methanolysis. The correlation between the methanolysis reaction conditions and FAME content was supported by a quadratic model, with ANOVA results indicating significant effects of reaction time and catalyst concentration, while the methanol-to-HSO molar ratio showed no significant impact. Optimal reaction conditions (methanol-to-HSO molar ratio of 6:1, catalyst concentration of 6.8% and reaction time of 45 min) yielded a predicted FAME content of 100.6%, closely matching the experimentally determined value of 99.1%.

The selected physicochemical properties of the obtained biodiesel, apart from the iodine value, complied with EN 14214 biodiesel quality standard. These findings highlight HSO as a promising feedstock for biodiesel production, reducing reliance on edible oil resources. The use of low-cost, non-toxic quicklime enhances the process's economic and environmental viability. However, further research on catalyst reusability, leachability, and a comprehensive techno-economic analysis is necessary to assess the potential for process commercialization.

Nomenclature

Abbreviations

TAG	Triacylglycerols
HSO	Hemp seed oil
FFA	Free fatty acid
FAME	Fatty acid methyl esters
RSM	Response surface methodology
HPLC	High-pressure liquid chromatography
ANOVA	Analysis of variance
MAG	Monoacylglycerols
DAG	Diacylglycerols
MRPD	Mean relative percentage deviation

Symbols

A	Catalyst concentration (%)
B	Methanol-to-HSO molar ratio (mol/mol)
b_o	Constant regression coefficient
b_i	Linear regression coefficient
b_{ii}	Quadratic regression coefficient
b_{ij}	Regression coefficient of two–factor interactions (i= 1, 2, 3 and $i<j\leq3$)
C	Reaction time (min)
R^2	Coefficient of determination (1)
R_{adj}^2	Adjusted coefficient of determination (1)
R_{pred}^2	Predicted coefficient of determination (1)
C.V.	Coefficient of variation
Y	FAME content (%)

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Izvod

OPTIMIZACIJA PROIZVODNJE BIODIZELA IZ ULJA SEMENA KONOPLJE (*CANNABIS SATIVA* L.) PRIMENOM NEGAŠENOG KREČA KAO KATALIZATORA

Biljana S. Đorđević, Milan D. Kostić, Marija R. Miladinović, Olivera S. Stamenković

Tehnološki fakultet u Leskovcu, Univerzitet u Nišu, Leskovac, Srbija

(ORIGINALNI NAUČNI RAD)
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Rad je fokusiran na proizvodnji biodizela iz nejestivog ulja semena konoplje (USK) primenom negašenog kreča, ekološki prihvatljivog katalizatora niske ekonomske vrednosti, što doprinosi razvoju održive proizvodnje biodizela. USK je dobijeno hladnim presovanjem semena, a najzastupljenije masne kiseline u njemu su linolna (55,70%), linoleinska (15,59%) i oleinska (13,72%) kiselina. Zbog povećanog sadržaja slobodnih masnih kiselina (SMK) u USK, primenjen je dvostepeni proces dobijanja biodizela: esterifikacija SMK katalizovana sumpornom kiselinom, a zatim metanoliza esterifikovanog ulja primenom negašenog kreča kao katalizatora. Reakcija metanolize je optimizovana primenom faktorijelnog plana 3^3 sa tri centralne tačke u kombinaciji sa metodologijom površine odgovora. Optimalni uslovi metanolize, pri kojima je postignut najveći sadržaj metil-estara masnih kiselina su bili: molski odnos metanol-USK 6:1, koncentracija katalizatora 6,8% i vreme reakcije 45 min. Fizičko-hemijske karakteristike dobijenog biodizela zadovoljavaju zahteve standarda kvaliteta biodizela EN14214, osim u pogledu jodnog broja.

Ključne reči: biodizel; metanoliza; negašeni kreč; optimizacija; ulje semena konoplje

Credit author statement
Biljana S. Đorđević: Conceptualization, Methodology, Investigation, Writing – Original Draft, Visualization
Milan D. Kostić: Conceptualization, Methodology, Investigation, Visualization, Writing – Original Draft
Marija R. Miladinović: Conceptualization, Validation, Writing – Original Draft
Olivera S. Stamenković: Writing – Reviewing and Editing, Validation, Supervision