

Valorization of *Griffonia simplicifolia* Seed Oil for Biodiesel Production: A Sustainable Alternative

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Abstract

Bio-derived oxygenated hydrocarbons, such as mixtures of fatty acid methyl esters (biodiesel), are promising alternatives for alleviating the adverse effects of fossil fuel consumption on climate change and preventing petroleum resource depletion. However, the selection of a viable feedstock for competitive biodiesel production remains challenging. Recent studies focusing on *Griffonia simplicifolia* seeds, the sole plant industrially exploited for 5-hydroxytryptophan (5-HTP) extraction, have shown that *G. simplicifolia* seed oil (GSO) can be solvent-extracted directly from ground seeds or the remaining seed cakes obtained after 5-HTP extraction with quantitative yields. This work documents the conversion of GSO into biodiesel through homogeneous base-catalyzed transesterification. The refractive index and density of the obtained methyl ester mixtures decreased with increasing oil-to-methanol molar ratio, reaction temperature, and time. Under specific conditions, 1.43 wt% FFA oil, 63.5°C, 60 min, and 1:9 oil/MeOH molar ratio with 1.2 wt% NaOH or 1.3 wt% KOH as catalysts, optimal reaction conditions were reached. There were no significant differences in the potential for diminution of the refractive index and density between the NaOH and KOH catalysts. The predicted fuel properties based on the fatty acid composition determined by GC-MS showed that *G. simplicifolia* biodiesel exhibited a cetane index of 50.29, volumetric energy density of 34.97 MJ/L, cloud point of -1.03°C, kinematic viscosity of 4.07 mm²/s, and oxidative stability of 0.65 h. Apart from its unfavorable oxidative stability and slightly lower energy density compared to petrodiesel, all other calculated parameters met the current standards. The valorization concept proposed in this study should be integrated into the 5-HTP extraction process, preferably using the remaining dry seed cakes as

raw materials to maximize revenue in a bioeconomic and sustainable approach.

Keywords

Griffonia simplicifolia, Seed Cakes, Vegetable Oil, Biodiesel

1. Introduction

The exacerbation of the consequences of climate change caused by anthropogenic greenhouse gas emissions is noticeable worldwide [1]. Among other factors, emissions from fossil fuel combustion [2], land-use changes, and forestry contribute to the amplification of positive radiative forcing. Simultaneously, concerns about unsteadiness in oil markets due to economic-geopolitical factors [3] and fossil resource depletion [4] are mounting. The previous scenarios spurred global interests in biofuels production processes such as the conversion of vegetable oils into fatty acids methyl esters mixtures, referred to as biodiesel, as sustainable alternative to petrodiesel [5].

However, non-cautious selection of proper feedstock for biodiesel production could disrupt agri-food chains and exacerbate land-use change.

Therefore, the valorization of by-products from plant origin within a circular economy approach represents a sustainable pathway capable of adding value to any production chain. The actual conceptual work focuses on the valorization of *Griffonia simplicifolia* seed oil into biodiesel.

Being the highest-concentrated natural resource in L-5-hydroxytryptophan (L-5-HTP), with contents up to 20% w/w [6], *G. simplicifolia* seeds are among the most valuable medicinal plants in West Africa as reported by the Ghana Investment Promotion Centre [7]. Thus, *G. simplicifolia* seeds are the most prolific and economically viable industrial source of 5-HTP [8], a molecule whose global market share was estimated at USD 51.6 million back to 2021 and projected to USD 101.0 million in 2030, with a compound annual growth rate of 7.82% [9]. 5-HTP is the precursor amino acid of serotonin, with applications ranging from cosmetics [8] [10] to nutraceuticals used as eco-compatible substitutes for synthetic neuroleptics such as Prozac, Paxil, Effexor, Luvox, and Zoloft [6].

Recent studies have highlighted that *G. simplicifolia* seeds contain oil up to ca. 31% which can be solvent-extracted with 92% of the theoretical yield [11] and pointed out that the remaining oil cakes still contains 5-HTP [12]. Furthermore, same researchers observed that the extraction of the oil and 5-HTP from the seeds was interchangeable. However, to the best of our knowledge there is no evidence in the literature regarding downstream valorization of the oil after 5-HTP extraction from the seeds. Therefore, to maximize the profitability of stakeholders in the trade and transformation of *G. simplicifolia* seeds into 5-HTP and its derivatives, it is essential to valorize the oleaginous potential of the cakes to add value to this industry.

This study aimed to determine the optimal conditions for methanol-induced homogeneous alkali-catalyzed transesterification of *Griffonia simplicifolia* seeds and predict some key fuel properties of the obtained biodiesel. The overarching goal is to contribute to the fight against climate change and add value to the export industry of *G. simplicifolia* seeds from West Africa to China, India, and other Western countries.

2. Material and Methods

2.1. Raw Materials

G. simplicifolia seeds served as plant materials for GSO extraction. The climbing shrub has orbicular and glabrous-shaped seeds in kidney-shaped pods (**Figure 1(a)**). At maturity, the pods split open, allowing for the collection of mature seeds from the floor. The seeds (**Figure 1(b)**) were harvested near the botanical garden of the Faculty of Sciences at the University of Lomé from December 2019 to March 2020. Afterward, the seeds were sun-dried, dehulled (**Figure 1(c)**), and grounded (grain diameter ≤ 2 mm).

In addition, crude palm kernel oil (PKO) served as a secondary raw material to compensate for the insufficient amount of *G. simplicifolia* seed oil that we extracted. The choice of PKO supplied by a cooperative was motivated by its abundance and affordability in the local markets in Togo. The optimal temperature and reaction time obtained during the transesterification of PKO were assumed to be identical for GSO conversion.

2.2. Extraction of *Griffonia Simplicifolia* Seeds Oil

The Soxhlet extraction of *G. simplicifolia* seed oil with hexane was performed four times using $m_o = 250$ g of seeds powder at each run. After 6 h, the solvent was evaporated using a Büchi rotary evaporator with the heating bath B-100 set at 40°C. Some residual hexane was definitively removed from the recovered oil fraction through oven-drying at 80°C for 2 h. The resulting oil was stored at -40°C for further use. The oil extraction yield, denoted as $Y(\%)$, was calculated using Formula (1).

$$Y(\%) = \frac{\text{mass}_{(\text{flask}+\text{oil})} - \text{mass}_{(\text{flask})}}{\text{mass of seeds powder}} \times 100 \quad (1)$$



Figure 1. *Griffonia simplicifolia* seeds ((a) = in non-mature pods; (b) = mature seeds with pericarps; (c) = dehulled mature seeds).

2.3. Preprocessing of Palm Kernel Oil

In the first step, the palm kernel oil (PKO) was settled down to decant, and the supernatant was removed and dehydrated using anhydrous Na_2SO_4 . Subsequently, the resulting product was oven-dried at $105^\circ\text{C} \pm 2^\circ\text{C}$ for 2 h before being hot-filtered. The recovered PKO was considered ready to transesterify and was stored at -40°C for further use.

2.4. Physicochemical Parameters and Fatty Acids Profile

2.4.1. Specific Gravity (SV), Acid Value (AV), Iodine Value (IV)

The specific gravity SG (15°C) was calculated using a correlation by Lund (Formula (2)) [13]. The refractive index (n_D^{20}) was determined using a binocular ABBE refractometer as reported by Kpoezoun *et al.* (2022) [14]. The acid value (AV) and free fatty acids content (%FFA), the saponification value (SV) (Formula (3)), and the iodine value (IV) (Formula (4)) were obtained experimentally using the conventional volumetric titration methods described by Kpoezoun *et al.* (2022) [14] and Gadegbe *et al.* (2019) [15]. Additionally, the fatty acid profile of GSO was determined using gas chromatography coupled with mass spectrometry (GC-MS) technique as described by Kpoezoun *et al.* (2022) [14]. Knowledge of fatty acids concentration in GSO allowed the calculation of the molecular weight (M_{GSO}) (Formula (5)) based on the formulae proposed by Halvorsen *et al.* (1993) [13]. The average molecular weight (\bar{M}_{PKO}) of PKO was calculated by correlating with the saponification value using Formula (7) suggested by Singhal and Kulkarni (1990) [16] and the molecular weight M_{PKO} was calculated using Formula (5). The ester percent (EP) was calculated using Formula (8) suggested by Canesin *et al.* (2014) [17]. Subsequently, the degree of unsaturation (DU), as defined by Ramos *et al.* (2009) [18], and was calculated using Formula (9). Finally, the calorific value was calculated using the correlation proposed by Demirbaş (1998) [19] (Formula (10)).

$$\text{SG}(15^\circ\text{C}) = 0.8475 + 0.00030\text{SV} + 0.00014\text{IV} \quad (2)$$

Given:

$$\text{SV} = \frac{56.104 \times 3 \times 100}{M_{\text{GSO}}} \quad (3)$$

$$\text{IV} = \frac{100 \times 126.9045 \times 2 \times 3 \times \text{DU}}{M_{\text{GSO}}} \quad (4)$$

2.4.2. Molecular Weight of GSO

$$M_{\text{GSO}} = 3 \times \bar{M}_{\text{GSO}} + 38.049 \quad (5)$$

Given:

$$\bar{M}_{\text{GSO}} = \frac{\sum \omega_{\text{FA}i}}{\sum \frac{\omega_{\text{FA}i}}{M_{\text{FA}i}}} \quad (6)$$

with \bar{M}_{GSO} , M_{GSO} , $\bar{M}_{\text{FA}i}$ and $\omega_{\text{FA}i}$: average molecular weight of fatty acids,

molecular weight of GSO triglycerides, molecular weight and mass fraction of fatty acid i , respectively.

2.4.3. Average Molecular Weight of PKO Fatty Acids

$$\bar{M}_{\text{PKO}} = \frac{56000}{\text{IV}} - 12.67 \quad (7)$$

$$\text{Ester Percent (\%)} = \frac{\text{IV} - \text{IA}}{\text{IV}} \times 100 \quad (8)$$

$$\text{DU (\%)} = \sum \% \text{MUFA} + 2 \sum \% \text{PUFA} \quad (9)$$

$$\text{PCS (MJ/kg)} = 49.43 - 0.041\text{SV} - 0.015\text{IV} \quad (10)$$

2.5. Transesterification Methodology

The triglycerides in PKO and GSO were reacted with methanol under base-catalyzed conditions using NaOH or KOH. The reaction vessel temperature was regulated using a TERMAKS/Model B 2324 V incubator system ($T_{\text{max}} = 64^\circ\text{C}$), and permanent mixing was achieved using a magnetic stirrer (VWR model 320) with a rotation speed set at 300 rpm. A thermometer was used to ensure the proper temperature of the pre-heated oil before adding the catalytic mixture (MeOH + NaOH or KOH).

2.5.1. Catalyst Amount

The mass of catalysts (NaOH or KOH) was calculated using Formula (11) and Formula (12) suggested by Van Gerpen *et al.* (2004) [20].

$$m_{\text{NaOH}} = ([\% \text{FFA}] \times 0.144 + 1\%) \times m_{\text{oil}} \quad (11)$$

$$m_{\text{KOH}} = ([\% \text{FFA}] \times 0.197/0.85 + 1\%) \times m_{\text{oil}} \quad (12)$$

with:

m_{oil} : mass of oil sample (PKO or GSO)

0.85: purity of KOH

Formula (11) and Formula (12) took into account the purity of the catalysts used, considering the total amount of catalyst as equal to the amount of alkali required to neutralize the free fatty acids plus 1% of alkali for effective catalysis of the reaction.

2.5.2. Volume of Methanol

The volume of methanol (V_{MeOH}) required for dissolving the catalyst was calculated using Formula (13).

$$V_{\text{MeOH}} = \frac{32.04 \times n_{\text{MeOH}} \times m_{\text{oil}}}{\text{Molecular weight of oil} \times \rho_{\text{MeOH}}} \quad (13)$$

with:

32.04 g/mol: molecular weight of methanol

n_{MeOH} : number of moles of methanol (see ratio)

ρ_{MeOH} : density of methanol

2.6. Post-Processing: Washing and Purification

After the reaction, the resulting mixture was transferred to a separating funnel. After 60 minutes, two immiscible phases were formed in the separating funnel. Then, the lower phase, mainly composed of glycerol, excess catalyst, altered pigments, etc. was separated from the FAME phase. Subsequently, the FAME phase was washed in multiple runs with near-boiling distilled water (water volume = 1/3 volume of the FAME phase). A colorless washing effluent upon adding phenolphthalein indicated a catalyst-free FAME mixture. The purification step of the previous FAME mixture involved residual methanol and free water removal by evaporating at $103 \pm 2^\circ\text{C}$ for 20 minutes, dehydration with Na_2SO_4 , and hot-filtration.

2.7. Optimization of PKO Transesterification Reaction

The one-factor-at-a-time method was adopted for optimizing the PKO-to-methanol (PKO/MeOH) molar ratio, the reaction time, and the reaction temperature. The experimental conditions are presented in **Tables 1-3**, respectively.

2.8. Optimization of GSO Transesterification Reaction

The optimal GSO-to-MeOH molar ratio and the influence of the catalyst type (NaOH or KOH) were investigated. The tested molar ratios were 1:6, 1:9, and 1:12. For each run, the mass of GSO used was 20 g; the required weights of NaOH

Table 1. Optimization of PKO-to-MeOH molar ratio.

Parameters	Experiments						
	E1	E2	E3	E4	E5	E6	E7
Mass of PKO (g)	20	20	20	20	20	20	20
Volume of MeOH (V_{MeOH} (mL))	0.0	3.2	6.4	9.6	12.8	15.9	19.2
PKO/MeOH molar ratio	1:0	1:3	1:6	1:9	1:12	1:15	1:18
NaOH catalyst amount (% w/w)	1.95	1.95	1.95	1.95	1.95	1.95	1.95

Constants: mass of PKO = 20 g; temperature $T = 60^\circ\text{C}$, reaction time $t = 100$ min and catalyst amount = 1.95% NaOH (w/w).

Table 2. Optimization of the reaction time.

Parameters	Experiments						
	E1	E2	E3	E4	E5	E6	E7
Mass of PKO (g)	20	20	20	20	20	20	20
Molar ratio PKO/MeOH	1:12	1:12	1:12	1:12	1:12	1:12	1:12
NaOH catalyst amount (% w/w)	1.95	1.95	1.95	1.95	1.95	1.95	1.95
Reaction time t (min)	0	20	40	60	80	100	120

Constants: Mass of PKO = 20 g; PKO/MeOH molar ratio = 1:12; Temperature $T = 60^\circ\text{C}$ and catalyst amount = 1.95% NaOH (w/w).

Table 3. Optimizing the reaction temperature.

Parameters	Experiments					
	E1	E2	E3	E4	E5	E6
Mass of PKO (g)	20	20	20	20	20	20
PKO/MeOH molar ratio	1:12	1:12	1:12	1:12	1:12	1:12
Reaction time t (min)	60	60	60	60	60	60
NaOH catalyst amount (% w/w)	1.95	1.95	1.95	1.95	1.95	1.95
Reaction temperature T (°C)	40.0	45.0	50.0	55.0	60.0	63.5

Constants: Mass of PKO = 20 g, PKO/MeOH molar ratio = 1:12; reaction time $t = 60$ min; catalyst amount = 1.95% NaOH (w/w).

or KOH catalysts were calculated using Formula (11) and Formula (12), respectively. The reaction temperature and time were identical to their optimal values determined for PKO, therefore set at $T = 63.5^\circ\text{C}$ and $t = 60$ min, respectively.

2.9. Qualitative Monitoring of FAME Yield

The qualitative monitoring of FAME formation has been performed by measuring changes in density and refractive index as a function of the kinetic parameters mentioned earlier, in reference to previous works by Froehner *et al.* (2007) [21] and De Filippis *et al.* (1995) [22].

2.10. Fuel Properties Prediction for GSO

Assuming that each triglyceride was converted into methylesters, some fuel properties of *G. simplicifolia* biodiesel, namely: the cetane number (CN), the kinematic viscosity (ν), the higher heating value (HHV), the volumetric energy density, the oxidative stability (OSI), and the cloud point (CP) were predicted exploiting GSO fatty acids composition and using identical mathematical models as those suggested by Talebi *et al.* (2014) [23] in their online graphical user interface BiodieselAnalyzer 2.2.

2.10.1. Cetane Number

The cetane number (CN) of a fuel is the volumetric percentage of n-hexadecane in a model blend of n-hexadecane and 1-methylnaphthalene that exhibits the same ignition delay as the sample tested [24]. CN was calculated using Formula (14) suggested by Ramírez-Verduzco *et al.* (2012) [25].

$$\text{CN} = -7.8 + 0.302 \cdot M_{FEi} - 20 \cdot N_{FEi} \quad (14)$$

Given:

M_{FEi} : molecular weight of the i th methylester

N_{FEi} : number of C=C bonds in the i th methylester

2.10.2. Kinematic Viscosity

The kinematic viscosity (ν) of a fluid is a measurement of its resistance to flow under a shear stress. In this study, ν was theoretically calculated using Formula

(15) as proposed by Ramírez-Verduzco *et al.* (2012) [25].

$$\ln(\nu) = \sum \omega_{FEi} \ln(-12.503 + 2.496 \times \ln(M_{FEi}) - 0.178 \times N_{FEi}) \quad (15)$$

with:

ω_{FEi} : mass fraction of the *i*th methylester

M_{FEi} : molecular weight of the *i*th methylester

N_{FEi} : number of C=C bonds in the *i*th methylester

2.10.3. Higher Heating Value and Energy Density

The higher heating value (HHV) of *G. simplicifolia* biodiesel was estimated theoretically based on the number of double bonds C=C and the molecular weight of each methylester using the Formula (16) suggested by Ramírez-Verduzco *et al.* (2012) [25], while the volumetric energy density (ED) was calculated by taking the product of HHV and the density (Formula (17)).

$$\text{HHV} \left(\frac{\text{MJ}}{\text{kg}} \right) = \sum \omega_{FEi} \left(46.19 - \frac{1794}{M_{FEi}} - 0.21N_{FEi} \right) \quad (16)$$

$$\text{ED} \left(\frac{\text{MJ}}{\text{L}} \right) = \text{HHV} \times \sum \omega_{FEi} \left(0.8463 + \frac{4.9}{M_{FEi}} + 0.0118N_{FEi} \right) \quad (17)$$

2.10.4. Oxidative Stability

The oxidative stability index (OSI) is the measurement of the induction period of a fuel sample. It has been demonstrated that allylic and bis-allylic positions in the mono and poly unsaturated fatty acids present in oils and resulting biodiesels are the preferred positions for the initiation and propagation of oxidation [26]. The calculation of OSI was performed based on the correlation of Knothe & Dunn (2003) [27] (Formula (18)).

$$\text{OSI}(h) = 3.91 - 0.045 \text{BAPE} \quad (18)$$

with $\text{BAPE} = C_{18:2} + 2 \times C_{18:3}$

$C_{x,y}$: Percentage of the unsaturated fatty acids $C_{x,y}$, BAPE: Bis-Allylic Position Equivalent.

2.10.5. Cloud Point

The cloud point (CP) represents the lowest temperature of the biodiesel at which the first “crystals” formed upon cooling become visible to the naked eye (diameter $\geq 0.5 \mu\text{m}$). CP was assumed to be a linear function of the quantity of palmitic acid and calculated using Formula (19) suggested by Sarin *et al.* (2009) [28].

$$\text{CP} (^{\circ}\text{C}) = 0.526 \times \% C_{(16:0)} - 4.992 \quad (19)$$

with $C_{(16:0)}$ the percentage of palmitic acid in the oil.

2.11. Statistical Analyses and Reproducibility

Each value in this study was presented as the mean of the measurements from two trials plus the standard deviation of the mean. The line plots were generated using Python 3 Matplotlib library. For comparison of the catalytic effect of

NaOH or KOH, a multiple t-test was performed using GRAPHPAD 8.4.3. If p-value > 0.01, the difference was considered non-significant, and significant otherwise.

3. Results and Discussion

3.1. Physicochemical Parameters of Feedstock Oils

G. simplicifolia seed oil and palm kernel oil physicochemical parameters are summarized in **Table 4**.

GSO extraction yield

The extraction yield of *G. simplicifolia* seed oil was $28.06\% \pm 0.38\%$. This yield was similar to the average values reported by Novidzro *et al.* (2019a) [11] and Giurleo (2017) [29], respectively 28.40% and 27% - 32%. Compared to soybean bearing an oil content ranging between 18% - 21% [30], *G. simplicifolia* seeds with an estimated oil content of 30.72% (Novidzro *et al.*, 2019a) [11] showcase an attractive oleaginous potential to be valorized in downstream processes to 5-HTP extraction. Therefore, botanical studies are needed to harness the reproduction of the plant. Besides, technical and economic analyses are required to assess the profitability of such processes.

Refractive index

The refractive index (n_D^{20}) measured for was 1.4651 ± 0.0001 . This value was lower than 1.4715 ± 0.0011 found by Novidzro *et al.* (2019a) [11] in a previous

Table 4. Physicochemical parameters of GSO and PKO

Parameters	GSO.	PKO
Oil extraction yield	$28.06\% \pm 0.38\%$	NA
Physical properties		
Refractive index (n_D^{20})	1.4651 ± 0.0001	ND
Specific gravity (15 °C)	0.92287	ND
Higher heating value (MJ/kg)	39.57	ND
Chemical properties		
Acid value (mg KOH/g)	2.86 ± 0.00	18.01 ± 0.08
% FFA (w/w)	1.43 ± 0.00	6.43 ± 0.02
Saponification value (mg KOH/g)	$172.59 \pm 1.87^a/192.21^b$	221.17 ± 0.20
Ester percent (%)	98.34 ± 0.02	91.86 ± 0.02
Iodine value (g I ₂ /100 g)	$25.58 \pm 0.45^c/132.23^d$	ND
Degree of unsaturation (% m)	152	ND
Molecular weight (g/mol)	875.68	759.64

^aIV: determined experimentally using Wijs method; ^bIV: predicted exploiting GSO fatty acids profile (Formula (4)); ^aSV determined experimentally using volumetric titration; ^bSV: predicted exploiting GSO fatty acids profile (Formula (3)); ND: Non-Determined; NA: Non-applicable.

study. It is frequently verified that (n_D^{20}) increases with the degree of unsaturation and the length of fatty acids lateral chain. In this study, the simultaneous effect of the higher concentration of C18 fatty acids and the higher degree of unsaturation (152%) of our GSO compared with that of Novidzro *et al.* (2019a) [11] would imply a higher (n_D^{20}) for our GSO. Such discrepancy means that other factors such as the unsaponifiable fraction would have much more influence on (n_D^{20}).

Specific gravity

The specific gravity at 15°C of the extracted GSO calculated using the equation of Lund gave 0.92368, corresponding to a density at 15°C of 0.92287 g/mL. This density exceeded the maximum range recommended for biodiesel, which is 0.860 g/mL to 0.900 g/mL [31]. This result indicated that neat GSO must be transesterified prior to possible usage in diesel engines.

Higher heating value

The estimated HHV for GSO was 39.57 MJ/kg. This value was in the same range as those reported in the literature for regular feedstocks used in biodiesel production, such as castor oil (39.79 MJ/kg), soybean oil (39.64 MJ/kg), sunflower seed oil (39.59 MJ/kg), and linseed oil (39.34 MJ/kg) [19].

Acid value and concentration of free fatty acids

Using volumetric titration, GSO and PKO acid values were 2.86 ± 0.00 mg KOH/g and 18.01 ± 0.08 mg KOH/g, respectively. These values corresponded to free fatty acid percentages of 1.43% (w/w) and 6.43% (w/w), respectively. For GSO, the obtained value was below the tolerable threshold of 2.5% (w/w) indicated by Musa (2016) [32] as safe for alkaline homogeneous transesterification. However, this acidity was higher than the 0.5 (% w/w) value mentioned by Freedman *et al.* (1984) [33] for achieving maximum methylesters yield (>98%). But, with preliminary dehydration, indirect heating, and moderate stirring rate, side reactions like saponification are less likely to occur. In the case of PKO, due to its high free fatty acids content, species presenting surfactant behavior are more likely to form during its conversion into biodiesel. Therefore, heterogeneous acid catalysis would be the preferred method of conversion.

Saponification value

The determined SV for our GSO, 172.59 ± 1.32 mg KOH/g was lower than the 186.59 ± 0.63 mg KOH/g previously obtained by Novidzro *et al.* (2019a) [11]. This difference is due to the higher C18 fatty acids content in our GSO (92.21%) compared to 87.88% from Novidzro *et al.* (2019a) [11] study. This difference also resulted in an approximate ten-unit difference in molecular weight. As for PKO, the obtained SV of 221.17 ± 0.14 mg KOH/g was below the range of 240 mg KOH/g - 257 mg KOH/g reported by some authors for palm kernel oils [34]. With respect to the latter, the transesterification of PKO would result in a lower methylesters yield compared to GSO, because the higher the SV of the oil, the lower methyl esters yield is obtained after base-catalyzed transesterification. Furthermore, additional precautions should be taken during the washing step of

PKO biodiesel, because this type of oil tends to easily give tensioactive molecules in alkaline conditions, which will act as emulsion stabilizers, thus hindering the aqueous and organic phase proper separation.

Ester percent

The ester percent is a metric for evaluating the quality of a raw material that will undergo homogeneous base-catalyzed transesterification. In this study, EP values of $98.34\% \pm 0.02\%$ and $91.86\% \pm 0.02\%$ were determined for GSO and PKO, respectively. Referring to the previous values, only GSO could provide a FAME yield greater than 98%, as Canesin *et al.* (2014) [17] reported a minimum of 96.5% glycerides to achieve a yield greater than 98%.

Iodine value

The experimental IV of our GSO was 25.58 ± 0.32 g I₂/100g of oil. This result was in a similar range as the IVs of palm kernel oils from the literature (15 - 23 g I₂/100g) and consistent with the previous determination by Novidzro (2019a) [11] classifying GSO as a non-drying oil. However, the theoretically calculated IV and DU based on GC-MS data were 132.23 g I₂/100g and 152.07%, respectively. The previous demonstrated considerable difference between theoretical and experimental values. The theoretical IV appeared more plausible considering the total amount of unsaturated fatty acids (UFA) in GSO, namely 79.50%. Additionally, the DU of our GSO, 152.07, was quite comparable to that of other oils with a similar unsaturated fatty acids composition, such as sunflower oil and soybean oil, which have DU values of 152.2% and 143.8%, respectively, with respective IVs of 132 g I₂/100g and 128 g I₂/100g [18]. If the theoretical result were given superior credit, it would imply that GSO is a drying oil since its IV is greater than 110 g I₂/100g. In the latter scenario, the IV of GSO significantly exceeded the maximum value (IV_{max} = 120 g I₂/100g) recommended by the European standard EN-14214 for feedstocks suitable for biodiesel production [35]. Furthermore, the theoretical degree of unsaturation of GSO surpassed the threshold indicated in European specifications, DU_{max} = 137 [18]. Finally, further investigation is required to explain conclusively the substantial gap between the experimental and theoretical results.

3.2. Fatty Acids Profile of *G. simplicifolia* Seeds Oil

The GC-MS analysis of the fatty acids composition of GSO provided the results summarized in **Table 5**. As per analysis results, GSO presents a high level of linoleic acid of 72.56%. The latter content was similar to the 73.19% reported by Novidzro *et al.* (2019c) [36]. Moreover, the aggregate percentage of unsaturated fatty acids (UFA), including linoleic acid (72.56%), oleic acid (6.93%), palmitoleic acid (0.01%), and (Z)-hexadec-11-enoic acid (0.01%) in our GSO, was 79.51%.

Other vegetable oils, such as safflower oil and grape seed oil, also shows similar linoleic acid percentage [37]. In the present GSO, palmitic and stearic acids were the predominant saturated fatty acids, with 7.54% and 12.72%, respectively.

Table 5. Fatty acids composition of *G. simplicifolia* seed oil.

Fatty acids	Percentage (%)	Molecular weight (g/mol)
Myristic acid	0.09	228.37
Palmitic acid	7.54	256.42
Stearic acid	12.72	284.48
Palmitoleic acid	0.01	254.41
Oleic acid	6.93	282.46
Linoleic acid	72.56	280.45
Isoheptadecanoic acid	0.06	270.45
13-Methyltetradecanoic acid	0.01	242.40
Hexadec-11-enoic acid	0.01	254.41
Others	0.02	-
¹ ND	0.04	-
² MUFA	6.95	-
³ PUFA	72.56	-

¹ND: Non-Determined, ²MUFA: Monounsaturated Fatty Acid, ³PUFA: Polyunsaturated Fatty Acid.

The previous palmitic and stearic acids contents in our GSO were in equal range as the values reported by other authors, ca. 9% - 11% for C16:0 and 16% - 18% for C18:0 [38] and 8.8%, 16.9% for C16:0 and C18:0, respectively [29]. The high cumulative amount of C18 fatty acids in GSO, ca. 92.21%, would favorably influence the cetane number (CN), as CN tends to increase when the aliphatic lateral chain length of fatty acids approach or exceed that of n-hexadecane. Conversely, the high content of unsaturated fatty acids (UFA), with a total amount of 79.51%, would adversely affect CN value, as CN decreases when the degree of unsaturation (DU) increases [39]. Moreover, the high UFA content is beneficial for the viscosity, as the latter tends to decrease with the increasing DU of the oil [40]. Similarly, the relatively low content of saturated fatty acids (SFA) in our GSO (20.42%) should result in more advantageous cold flow properties.

3.3. Optimization of PKO Transesterification

In this study, we qualitatively accessed the yield of FAME after each PKO sample transesterification experiments by measuring the variation in density and refractive index of the resulting transesterified mixtures. The data points obtained allowed the construction of the line plots depicted in **Figures 2(a)-(c)**.

It is worth mentioning that the density values used to establish the line plots were measured without accounting the effect of air buoyancy. Therefore, in this section the density implies non-corrected density ρ_{nc} .

With respect to the FFA content of the PKO used in this study (6.43%), the optimal reaction conditions for the transesterification reaction, were: optimal

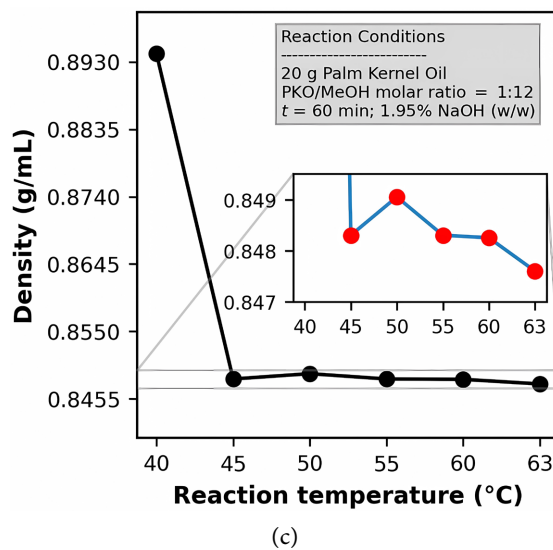
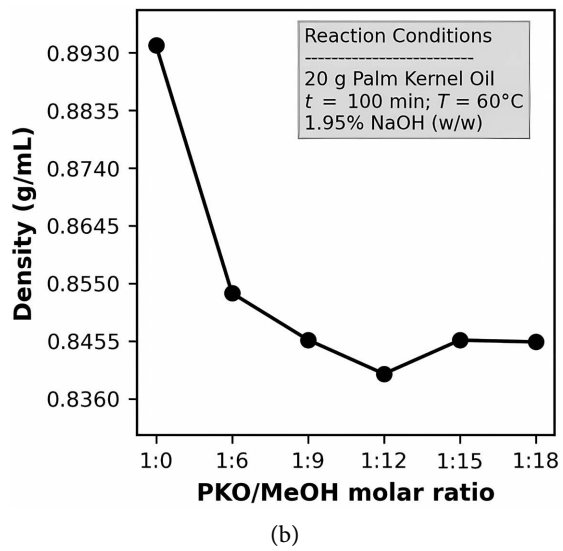
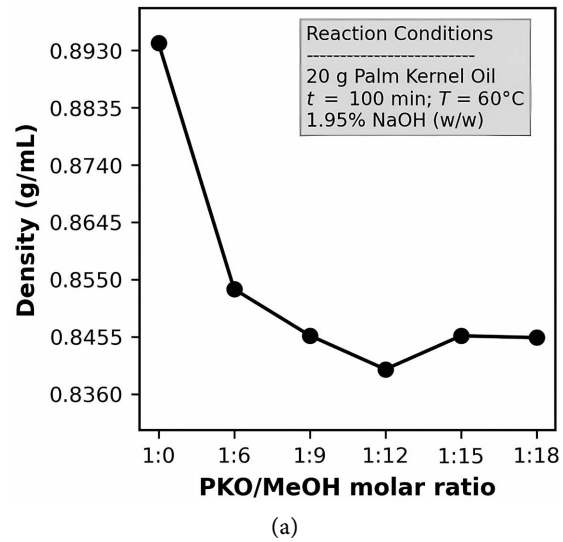


Figure 2. (a) Density vs Molar ratio of PKO; (b) Density vs reaction time PKO; (c) Density vs reaction temperature PKO.

reaction temperature $T_{opt} = 63.5^{\circ}\text{C}$, optimal reaction time $t_{opt} = 60$ min, and optimal PKO/MeOH molar ratio $\text{MR}_{opt} = (1:12)$. Following each experiment, a diminution in the value of the density of the formed FAME mixtures compared to the parent oil was observed versus increasing reaction parameters values, either PKO/MeOH molar ratio (**Figure 2(a)**) or reaction time (**Figure 2(b)**) or reaction temperature (**Figure 2(c)**). The density ρ_{nc} decreased from the maximum value of 0.8942 g/mL (neat PKO) to a minimum of 0.8401 g/mL as PKO/MeOH molar ratio increased from (1:3) to (1:12). Beyond a molar ratio exceeding $\text{MR} = 1:12$, the opposite effect occurred. In particular, at $\text{MR} = 1:3$, the obtained reaction mixture solidified in the separating funnel. Such an observation is due to the higher rate of the saponification reaction of glycerides compared to that of the transesterification reaction.

The reduced density of the transesterified product compared to that of the parent oil is due to the progressive substitution of the glycerol moiety in glycerides by methanol molecules. Since pure glycerol is denser than neat vegetable oil, its cleavage out of the medium consequently leads to a decrease in the density of the resulting mixture. However, an excessive excess of methanol resulted in poor separation of the FAME phase from the glycerol phase leading to a decrease in FAME yield. The presence of diluted glycerol in the FAME phase leads to the apparent loss of methyl esters due to micelles formation [32]. Additional explanation suggested by Phan & Phan (2008) [41] assumed a reduction in the rate of conversion of triglycerides into FAMEs. According to the latter hypothesis, one must first consider the mechanism through which excess methanol would initially lead to a high conversion of glycerides into FAME. Afterward, as more FAME molecules are formed, consequently increasing amount of glycerol is generated. Ultimately, as the reaction medium approaches saturation in glycerol, the excess glycerol molecules in the medium would automatically shift the equilibrium to the reverse side in compliance with the laws of thermodynamic equilibria.

3.4. Optimization of GSO Transesterification Reaction

The line plots obtained by measuring the density and refractive index of transesterified mixtures at selected molar ratios depending on the catalyst type (NaOH or KOH) are depicted in **Figure 3**, **Figure 4** and **Table 6**. Regardless of catalyst type, GSO/PKO $\text{MR} = 1:9$ resulted in the FAME mixtures with optimal density and refractive index, thus predictively optimal FAME yield. Expectedly, the refractive index of the transesterified mixtures decreased as the oil-to-methanol molar increased (**Figure 5** and **Table 6**). In other words, the refractive index of transesterified vegetable oils decreases as the FAME yield increases, in compliance with the results of Xie & Li (2006) [42]. Additionally, the paired-comparison plots comparing KOH or NaOH capability in lowering the refractive index and the density of parent oils are illustrated in **Figure 5** and **Figure 6**. According to the latter results, there was no significant differences in the catalytic

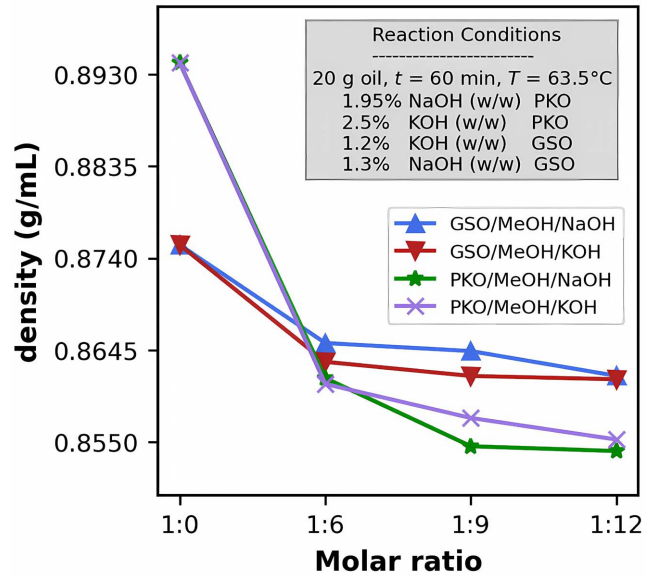


Figure 3. Density vs molar_ratio_GSO_PKO.

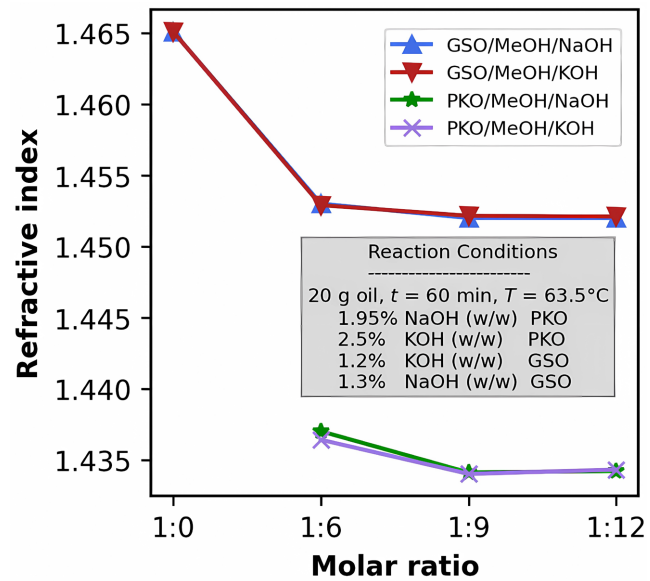


Figure 4. Refractive index vs molar_ratio_GSO_PKO.

Table 6. Variation in density and refractive index versus oil-to-MeOH molar ratio.

MR	Catalysts	GSO				PKO			
		NaOH		KOH		NaOH		KOH	
Parameters		n_D^T	ρ_{nc}	n_D^T	ρ_{nc}	n_D^T	ρ_{nc}	n_D^T	ρ_{nc}
1:0		1.4641	0.87539	1.4641	0.87539	---	0.89420	---	0.89420
1:6		1.4530	0.86524	1.4529	0.86330	1.4370	0.86162	1.4364	0.86101
1:9		1.4520	0.86441	1.45215	0.86184	1.4341	0.85458	1.4340	0.85750
1:12		1.4520	0.86185	1.4521	0.86150	1.4342	0.85408	1.4343	0.85527

n_D^T : refractive index; ρ_{nc} : density.

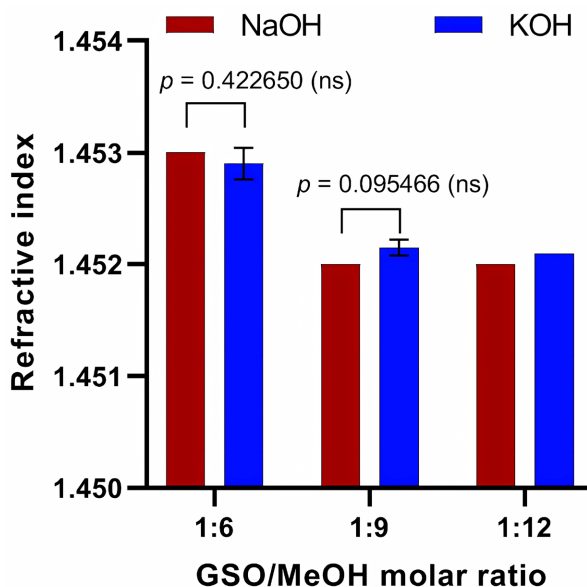


Figure 5. Barchart refractive index vs GSO_MeOH molar ratio.

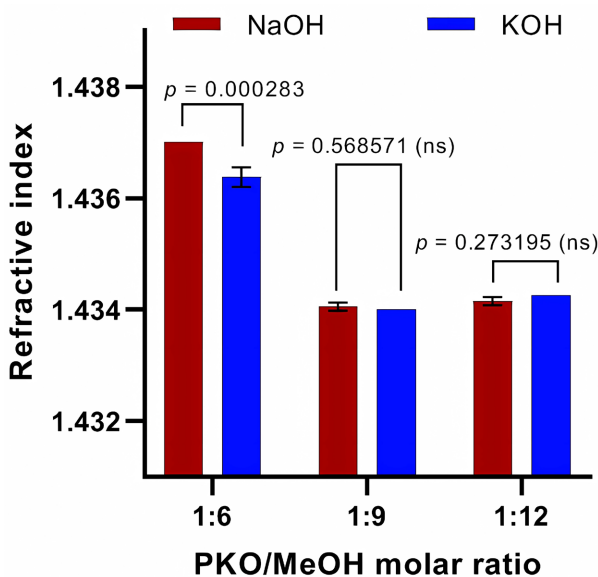


Figure 6. Barchart refractive index vs PKO molar ratio.

performance of NaOH or KOH, either during GSO ($p = 0.1449 > 0.05$) or PKO ($p = 0.5240 > 0.05$) conversions. Many attempts at establishing a linear regression relationship between the refractive index and the yield of alkylesters as a rapid method of verification in continuous biodiesel production are available in the literature [43] [44] [45].

Finally, in the present study the preferred optimal reaction conditions for the transesterification of GSO were as follows: 1.3% KOH (w/w); GSO/MeOH molar ratio $MR_{opt} = 1:9$; reaction time $t_{opt} = 60$ min, and temperature $T_{opt} = 63.5^\circ\text{C}$. There is however, a need of quantitative instrumental techniques such as Gas Chromatography (GC) or High-Performance Liquid Chromatography (HPLC)

to measure the FAME yield and validate our assertions.

3.5. Fuel Properties Prediction of GSO Biodiesel

The physicochemical parameters of GSO biodiesel calculated using various linear regression correlations and relying upon data from the fatty acid composition of GSO are compiled in **Table 7**.

The calculated OSI of GSO biodiesel resulted in an induction period of 0.65 hours. This induction period did not comply with the EN 14214 (3 h) and ASTM 6751 (6 h) specifications. According to previous work by Kumar (2017) [46], GSO biodiesel would be classified as a biodiesel with poor oxidative stability, since $OSI < 2$ h. Therefore, according to Kumar (2017) [46], this type of biofuel would require a significant amount of antioxidant additives, regardless of specifications, to achieve the recommended quality, thus incurring higher costs. Being predominantly unsaturated ($DU \approx 152$), the bis-allylic positions $\Delta 11$ of polyunsaturated fatty acids (PUFA) and allylic positions $\Delta 11$ of monounsaturated fatty acids (MUFA) will have a strong tendency to undergo homolytic cleavage under oxidative conditions. As a result, it is imperative to implement efficient storage conditions to isolate GSO and resulting biodiesel from ambient air, light, metals, or other pro-oxidative factors.

The higher heating value (HHV) calculated for GSO biodiesel was 39.72 MJ/kg. The latter value is approximately 8% lower than that of No. 2 petrodiesel fuel [47]. Correspondingly, the previous value relates to a volumetric energy density of 34.97 MJ/L, which is considerably lower than that of petrodiesel, ca. 40 MJ/L [48]. Consequently, a volume of ca. 1144 mL of GSO biodiesel would be required to deliver equal energy output as 1 L petrodiesel, thus negatively affecting the pump prices. Therefore, to maximize the energy return on investment (EROI), it will be best to align this process with the valorization of niche molecules such as 5-HTP, lectins, and antioxidants in *G. simplicifolia* seeds.

Table 7. Predicted fuel properties of GSO biodiesel.

Properties	References	Values	Specifications	
			EN 14214	ASTM D 6751
SV (mg KOH/g)	[26]	191.30	-	-
IV (g I ₂ /100g)	[26]	132.90	120 max	
¹ CN	[25]	50.29	51 min	47 min
ρ (g/mL)	[25]	0.8803	-	0.8 - 0.9
² HHV (MJ/kg)	[25]	39.72	-	-
Energy density (MJ/L)	(The current study)	34.97		
³ CP (°C)	[28]	-1.03	-	-
⁴ ν (40°C) (mm ² /s)	[25]	4.07	3.5 - 5.0	1.9 - 6.0
⁵ OSI à 110°C (h)	[27]	0.65	6 h min	3 h min

¹CN: Cetane Number; ²HHV: Higher Heating Value; ³CP: Cloud Point; ⁴ ν : kinematic viscosity; ⁵OSI: Oxidative Stability Index.

The calculation of the cetane number (CN) of GSO biodiesel yielded 50.29. This value complies with ASTM D 6751 specification. Comparatively, the calculated CN value was close to those of soybean biodiesel (51.8) and sunflower biodiesel (51.9) [49].

The determination of the kinematic viscosity at 40°C of GSO biodiesel resulted in a value of 4.07 mm²/s. This value adhered to EN 14214 and ASTM D 6571 specifications. However, it significantly exceeded that of Ultra Low Sulfur Diesel, which is 2.32 mm²/s [50].

The predictive calculation of the cloud point of GSO biodiesel using the correlation suggested by Sarin *et al.* (2009) [28] gives a value of -1.03°C. Nonetheless, minor compounds in GSO, such as steryl glycosides, terpenoids, and phospholipids, can dramatically influence this parameter up. In reality, it is plausibly expectable that the experimental cloud point of GSO biodiesel might be higher than the theoretically predicted value depending on the presence and quantities of these minor compounds.

Apart from the poor oxidative stability (OS), each fuel property calculated in this study for GSO biodiesel complies with the European EN 14214 and the American ASTM D 6571 standards.

4. Conclusions

We took advantage of the energetic potential of *Griffonia simplicifolia* seeds oil in this study by converting it into biodiesel. To produce biodiesel, GSO was first extracted from ground mature seeds. The transesterification of the oil was carried out using NaOH and KOH catalysts. Among both methods that allowed qualitative monitoring of FAME yield, recording the diminution of the refractive index was more accurate and faster than measuring the decrease of the density. With a free fatty acid content of 1.43% (w/w), the optimal reaction parameters values were as follows: oil/methanol molar ratio = 1:9, reaction time = 60 min, temperature = 63.5°C, 1.2% NaOH (w/w) or 1.3% KOH (w/w), with a preference for KOH due to its easier dissolution in methanol. Under the optimized experimental conditions, KOH and NaOH exhibited similar effect in their ability to catalyze the decrease in density or refractive index. This study classified *G. simplicifolia* seed oil as a drying oil based on the theoretical results calculated for IV and DU. The significantly high DU of GSO resulted in a low induction period of 0.65 hours, indicating poor resistance of the oil and biodiesel to oxidation. Except for the OS, all other predicted fuel properties of GSO biodiesel met both EN 14214 and ASTM D 6571 standards.

Therefore, engine tests need to be carried out to evaluate the suitability of GSO biodiesel for usage in diesel engines.

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Conflicts of Interest

All authors of this manuscript declare that there are no conflicts of interest regarding the publication of this manuscript.

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Abbreviation

GSO: *Griffonia simplicifolia* seed oil

PKO: Palm kernel oil

FAME: Fatty acid methyl ester

5-HTP: 5-hydroxymethyltryptophane

SV: Saponification value

IV: Iodine value

CN: Cetane number