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Production of Less Controversial Biofuels: Case of Biodiesel from Unsuitable Palm Oil for Consumption

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Abstract

The climate crisis is one of today's major challenges. One of the main causes is the increasing use of fossil fuels, responsible for GHG emissions. To overcome this difficulty, enlightened researchers have come up with alternatives among which biofuels, particularly biodiesel, taking pride of place. Indeed, biodiesel is a clean, and renewable fuel, produced from vegetable or animal fats using transesterification process. This study aimed to optimize the transmethylation reaction of unsuitable palm oil into biodiesel. The oil physicochemical characterization was carried out according to AFNOR standards. Pretreatment of the crude oil was carried out at 60°C. Transmethylation of the pretreated oil was optimized based on three key parameters. The results showed that the FFA content of the oil exceeded the threshold required for direct transesterification by basic catalysis. Optimum conditions for oil pre-treatment were: molar ratio MeOH/FFA = 24/1, %H₂SO₄ = 10.27 and reaction time = 90 min. Optimization of the oil transmethylation reaction was achieved with MeOH/oil molar ratio =6/1, %KOH= 1.51 and reaction medium temperature = 60°C. Thanks to its low viscosity, the biodiesel produced could be an excellent alternative to diesel. However, it would be wise to carry out preliminary tests on diesel engines first.

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Introduction

The fast pace of industrial growth, coupled with a growing world population, has resulted in a growing need for energy. The main sources of energy currently being exploited in the world include petroleum, natural gas, coal, hydroelectricity and nuclear energy (Cvengroš and Cvengrošová, 2004; Kulkarni and Dalai, 2006). One of the major current issues linked to energy consumption in the world is the soaring price of

petroleum products (Mitiku *et al.*, 2020), with negative repercussions as consequence on household incomes, especially in countries that are not producers of petroleum. This is not the first time this energy crisis, known as the petroleum shock, since we had witnessed it recently in 2008, when the price of a barrel of oil rose to US\$145, and again in 2011, when it reached US\$108 (Chamoumi, 2013). Apart from skyrocketing prices for petroleum products, their use is also a source of

greenhouse gas (GHG) emissions as well as other pollutants.

The main GHG are carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (NO₂), with proportions of 75%, 18%, and 7% respectively in 1990 (Mikhaylov *et al.*, 2020a). Everybody knows that the heavy accumulation of GHGs in the atmosphere causes climate change, with its detrimental effects on aerial, terrestrial and aquatic ecosystems, leading to incalculable and unbearable environmental crises for living beings (Kweku *et al.*, 2018).

However, to reduce these GHG emissions, the use of environmentally-friendly energies such as biofuels (bioethanol, biodiesel, biogas and biohydrogen) would appear to be beneficial. Historically, the production of biofuels in general, and biodiesel in particular, has a long history. As far as biodiesel is concerned, its production had begun when Rudolph Diesel (1858-1913), inventor of the diesel engine, used peanut oil as the first source of energy for its functioning (Chamoumi, 2013). However, this first fuel for Diesel engine had been abandoned in favor of petroleum, thanks to its cheap availability.

Biodiesel is defined as clean, biodegradable and renewable energy produced from vegetable oils and animal fats. It is a highly promising bioenergy alternative to substitute diesel (Sharma *et al.*, 2014). Depending on its intrinsic chemical nature, a crude vegetable oil (CVO) or a crude animal fat (CAF) is mainly made up of triesters of fatty acid and glycerol called triglycerides (Morin and Pagès-Xatart-Parès, 2012).

Remember that CVO itself is a source of energy, but its use as a fuel causes problems of clogging and deposits in the engine, due to its high viscosity (Knothe, 2006). To overcome these problems, CVO would have to be chemically modified through the substitution reaction, which consists of replacing glycerol with short-chain alcohols to obtain what is eventually called biodiesel (Erchamo *et al.*, 2021).

Finally, the chemical nature of the biodiesel obtained is a mixture of fatty acid monoesters. Contrary to diesel, whose chemical composition consists exclusively of a mixture of hydrocarbons, biodiesel is essentially a mixture of oxygenated hydrocarbons (Hoekman *et al.*, 2012). The presence of oxygen atoms in the chains of biodiesel constituents has the advantage of greatly enhancing the complete combustion of biodiesel in engines, thus reducing emissions of unburnt particles

into the atmosphere (Lapuerta *et al.*, 2008). Moreover, during photosynthesis, the oil plants use CO₂ for their growth. It is therefore clear that the substitution of diesel by biodiesel is an effective means of reducing atmospheric CO₂, which alone is responsible for more than 75% of GHG emissions (Mikhaylov *et al.*, 2020b). So since the 1970s, with recurring energy crises, researchers around the world have been constantly looking for credible alternatives for fossil fuels (Chamoumi, 2013). Much of their researches has focused on the production of renewable energies, in particular biofuels such as bioethanol and biodiesel (Ahiekpor *et al.*, 2016; Ambaye *et al.*, 2021).

Regarding to the need for fighting against global warming, in this study we have decided to focus on the production of biodiesel, the most suitable biofuel for diesel engines. Indeed, our approach is to contribute to the valorization of unsuitable oils, as these inedible substances, when not used, may have negative impacts on the environment, such as water pollution and soil impoverishment.

This study aimed to develop the processes of optimizing biodiesel production from unsuitable vegetable oil for consumption.

Materials and Methods

Justification of raw material choice

The raw material used in the current work was palm oil, unsuitable for consumption. This oil was purchased in August 2021 in Keve (Ave-Togo District) from Ghana traders who were selling the oil from the Kumasi-Ghana region to Benin via Togo.

This oil's choice for biodiesel production is not accidental. Indeed, according to the information received from the sellers, the particular oil referred to in the current work was extracted from an improved variety of palm selected in Ghana, which admittedly has a high oil yield, but whose taste is not appreciated by the local population. For this reason, the oil was generally used only for soap production. However, as the supply was greater than demand, extended storage of this oil in retail outlets in the three above-mentioned countries was becoming a serious risk to their environments.

Furthermore, the cost of diesel is also becoming more and more exorbitant nowadays on national and international markets. In addition, the fight against global warming through the development of simple biofuel

production methods was also seen as a beneficial initiative supported by the international community. This explains our motivation to convert unsuitable palm oil (UPO) into biodiesel.

Physicochemical characterization of the UPO

The physicochemical parameters of UPO, such as: water and volatile matter content (M_C), density ($\rho^{20^\circ\text{C}}$), refractive index ($n_D^{20^\circ\text{C}}$), and saponification index (SI), acid value (AV), the free fatty acid percentage (%FFA) and ester percentage (EP) were determined experimentally according to AFNOR Standard volumetric dosage methods, reported by Kpoezoun *et al.*, (2022) and Gadegbe *et al.*, (2019).

However, the molar mass of the oil (M_O) was calculated theoretically from *Formula 1*, proposed by Zhu *et al.*, (2006).

$$M_O = \frac{56.1 \times 3 \times 1000}{(SI - AV)} \dots(1)$$

With:

SI : saponification index,

AV : acid value.

Pretreatment optimization the UPO

As the FFA content of the oil used as feedstock for biodiesel production exceeds the threshold required for direct transesterification by base catalysis in homogeneous phase, it was decided to pretreat the oil in order to optimize the biodiesel production yield.

Parameters considered in this work to optimize UPO pretreatment were: molar ratio of methanol to free fatty acids (MeOH/AGLs), pretreatment time and acid catalyst concentration. The strategy used was to vary just one parameter, while the others were kept fixed.

In Tables 1, 2 and 3, the variations in MeOH/FFA molar ratio, %H₂SO₄ and pretreatment time applied during acid-catalysis pretreatment are shown, respectively.

Optimizing the transmethylation reaction of the UPO

In this study, the three parameters whose variation was monitored in order to optimize the oil transmethylation reaction were: the MeOH/triglyceride molar ratio, the reaction time and the H₂SO₄, acid catalyst rate.

In Tables 4, 5 and 6, respectively, the variations in the MeOH/triglyceride molar ratio, %KOH and reaction medium temperature applied during optimization of the transmethylation reaction are shown.

Characterization of biodiesel produced

The density and refractive index of the biodiesel were the parameters measured according to AFNOR standards for the characterization of our biodiesel.

Results and Discussion

Physicochemical characteristics of UPO

The physicochemical characteristics of UPO used in the current work as a feedstock for biodiesel production are presented in Table 7.

Impact of the alcohol/FFA molar ratio on the esterification reaction

The histograms presented in Figure 1 showed the influence of the methanol/FFA molar ratio on the FFA content during UPO pretreatment.

Examination of the histograms in Figure 1 had revealed that the methanol/FFA molar ratio had a significant impact on the FFA content during UPO pretreatment. Indeed, it was observed that the FFA content decreased as the methanol proportion increased, starting from a methanol/FFA molar ratio of (8:1) and reaching a minimum value of (32:1) before rising again. Concomitant with this variation in the methanol/FFA molar ratio, FFA content decreased from 9.23% to 0.27% (Figure 1).

Thus, the maximum decrease in FFA content in the oil was observed for a methanol/FFA ratio of (32:1), corresponding to an equivalent methanol/oil molar ratio of (12:1). This decrease in FFA content was seen as evidence of the conversion of the FFAs contained in the oil into methyl esters, in that way supporting the use of the pretreated oil for direct transesterification by basic catalysis in the homogeneous phase.

Catalyst content influence on the FFA esterification reaction

The profile of FFA content in UPO as a function of the rate of H₂SO₄ (m/m) applied as catalyst during pretreatment was shown in Figure 2.

The curve in Figure 2 had shown that with acid (H_2SO_4) levels ranging from 0% to 15.76% applied during the pre-treatment process, there was a progressive decrease in the FFA content in the UPO, from a level of 9.23% up to the limit value of 0.97%. This subsequent decrease in FFA content was proportionally related to the catalyst concentration. Applying the optimum catalyst value of 10.87% (H_2SO_4) had allowed to reduce the FFA content in UPO yield of 89.49% (Figure 2).

Effect of time on the esterification of FFAs from UPO

In the experimental conditions involving reaction medium temperature, methanol/FFA molar ratio and catalyst ratio (% H_2SO_4) set at 65°C, (24:1) and 10.87% respectively, the esterification reaction was optimized according to time.

In Figure 3, the curve showing the variation of FFA content in UPO according to the time of the esterification reaction was presented.

The curve presented in Figure 3 below showed a progressive decrease in FFA content during the first 90 minutes before reaching equilibrium. Indeed, the FFA content had decreased from 9.23% marking the start of esterification to 0.5% showing the finally reached equilibrium state. This result had shown that a minimum duration about 90 minutes was required to esterify FFAs in UPO, with an approximate conversion rate of 98.9%.

Transmethylation reaction optimization

Influence of KOH content on the transesterification reaction

The influence of KOH on the transmethylation reaction of pretreated UPO was evaluated using the refractive index (RI) curve and the curve of fatty acid methyl esters (FAMES) that were formed. The evolution of RI methyl esters formed according to %KOH was illustrated in Figure 4.

According to this curve, the optimal KOH level deduced for the transmethylation of our UPO sample was close to 1.52%. With a percentage of KOH lower than 0.51%, the transesterification reaction failed, yielding a heterogeneous liquid mixture that was difficult to separate. In contrast, at KOH contents upper than the optimum value (1.52%), the reaction generated soap.

Influence of temperature on transesterification reaction

Figure 5 showed the RI variation of the FAMES formed according to the temperature of the reaction medium. As shown in Figure 5, the biodiesel with the lowest RI of 1.4485 was obtained at the optimum temperature of 60°C.

Influence of methanol/triglyceride molar ratio on transesterification

In order to investigate the influence of the methanol/triglyceride molar ratio on the transmethylation reaction of our pre-treated UPO, the histograms shown in Figure 6 were used.

From the data presented in Figure 6, the optimum value of the methanol/triglyceride ratio deduced was (6:1). This result was equivalent to the methanol/oil molar ratio of (5.34:1).

Refractive index and volumic mass of biodiesel of UPO

From the optimal conditions previously revealed in the current work, FAMES produced as biodiesel are characterized by IR and Vm, respectively equal to 1.4495 ± 0.0007 and 0.8595 ± 0.0007 g/mL (Table 8).

Physicochemical characteristics of UPO

Water and volatile matter content (Wc)

The water and volatile matter content (Wc) of our UPO, *i.e.* $0.15\% \pm 0.01\%$, was lower than the maximum value of 0.50% (Canakci and Gerpen, 1999), which must not be exceeded, otherwise the transesterification reaction will be unfavorably affected. In fact, it has been found that when Wc of an oil exceeds 0.50%, this leads to the formation of soap, *i.e.* saponification reaction.

Saponification is a parasitic reaction that hinders the transesterification of vegetable oils, thus reducing biodiesel production yields (Canakci and Gerpen, 1999). Experimentally, when Wc greatly exceeds 0.50%, this also favors the hydrolysis reaction of the esters formed in the biodiesel, with the major drawback of lower biodiesel production yields (Canakci and Gerpen, 1999).

Volumic mass

The value of volumic mass (V_m) of our UPO, 0.9005 ± 0.0007 g/mL, was lower than that of water. This value is in accordance with the specific characteristics of fats. In comparison, the value found for the volumic mass of our oil was much higher than that of diesel, which is about 0.850 g/mL (Inrets *et al.*, 2004). This shows that our oil cannot be used directly as a fuel in diesel engines due to the increased viscosity of crude oils which a parameter strongly linked to volumic mass (Daho *et al.*, 2008).

Refractive index

The refractive index (RI) of our UPO at 20°C was about 1.4653 ± 0.0006 . This value was almost the same as that found by Khan *et al.*, (2010), *i.e.* 1.465 for a mixture of palm oil and rubber seed oil.

Acid value and percentage of free fatty acids

The acid value (A_v) of UPO was about 20.22 ± 0.00 mg KOH/g oil, equivalent to a percentage of free fatty acids (%FFA) or acidity of 9.23% (m/m). This result showed that our oil was not suitable for consumption as its acidity exceeded the recommended threshold for edible oils, which is 5% (Japir *et al.*, 2017).

This oil conversion into biodiesel could then be considered as a new route for its valorization just like the one that already consisted in its transformation into soap by consumers. However, the method of direct transesterification of the oil by basic catalysis would not be feasible as the %FFA exceeded the set threshold of 2%.

Either the palm nuts were stored for a long time before extraction, or the oil was badly preserved after extraction, would certainly be at the root of the high FFA content observed. However, it had also been found that excessive water content could also cause hydrolysis of the esters contained in the oil, thereby leading to the formation of additional FFA. In a similar direction, other authors (Ali *et al.*, 2014; Frank *et al.*, 2011; Kumaradevan *et al.*, 2015; Ohimain *et al.*, 2013) had reported that the effect of lipases from wounded nuts engenders the hydrolysis of triglycerides, resulting in an increased acid value of the extracted oil.

Saponification value, ester percentage and molar mass of the UPO

The saponification value (S_v) of our palm oil was about 182.30 ± 0.01 mg KOH/g oil. This value was then below the range indicated for the S_v of palm oils, which is usually between 191 and 235 mg KOH/g of oil (Ohimain *et al.*, 2013). Considering that the S_v of a fatty substance is lower than the carbon chains of its fatty acid constituent (Novidzro *et al.*, 2019), we could suggest that our oil contains fatty acids with long carbon chains.

The value deduced for the ester percentage of the oil analyzed in the current work was about $88.91 \pm 05\%$. On the basis of the S_v and A_v obtained, simulation of the molecular molar mass of our UPO, deduced using the formula proposed by Zhu *et al.*, (2006), had given a value equivalent to 1038.39 g/mol. This simulated value thus appeared much higher than those for palm oils, between 800 and 900 g/mol (Thanongsak, 2007).

Impact of the alcohol/FFA molar ratio on the esterification reaction

However, a methanol/oil molar ratio of (9:1) was lower than that obtained in this study, that had enabled Bojan and Durairaj (2012) to reduce the FFA content of *Jatropha Curcas* oil from 6.85% to 1.12%. Hayyan *et al.*, (2014) also reported a maximum reduction in FFA content for a methanol/oil ratio of (10:1). In the literature, however, slightly higher methanol/oil molar ratios have been reported. For example, Encinar *et al.*, (2011) reported a methanol/oil molar ratio of (18:1). Similarly, Khan *et al.*, (2010) also used a methanol/oil molar ratio of (15:1) to minimize the FFA content in their sample.

The decrease in FFA content according to the augmentation of methanol volume would have a definite reason. Indeed, as the esterification reaction is reversible, this suggests that whenever one of the reactants is in excess (methanol in our case), the equilibrium is favorably shifted in the direction of product formation. However, the tendency for the phenomenon to stabilize or even increase in FFA content, as observed in our study, would be linked either to the accumulation of water from the esterification reaction (Encinar *et al.*, 2011), or to the decrease in catalyst concentration due to the significant increase in methanol volume.

Table.1 Variation in MeOH/FFA molar ratio during esterification pretreatment

Molar ratio of MeOH/AGLs	(8:1)	(16:1)	(24:1)	(32:1)	(40:1)	(48:1)	(56:1)
UPO weight (in g)	20	20	20	20	20	20	20
FFA weight (in g)	1.84	1.84	1.84	1.84	1.84	1.84	1.84
Methanol (mL)	2.32	4.65	6.97	9.30	11.62	13.95	16.27

Table.2 Variation in concentrated %H₂SO₄ during esterification pretreatment

UPO weight (in g)	20	20	20	20	20	20
FFA weight (in g)	1.84	1.84	1.84	1.84	1.84	1.84
% H ₂ SO ₄	1.09	1.63	2.17	5.43	10.87	15.76
Mass of concentrated H ₂ SO ₄ (g)	0.02	0.03	0.04	0.10	0.20	0.29

Table.3 Variation in the time of esterification pretreatment

Order numbers	1	2	3	4	5	6	7
Pretreatment time (min)	15	30	50	70	90	110	130

Table.4 Variation in the MeOH/triglyceride molar ratio tested during transmethylation

UPO weight (in g)	20	20	20	20	20	20
Triglyceride weight (in g)	17.78	17.78	17.78	17.78	17.78	17.78
MeOH weight (in g)	1.64	3.29	4.93	6.58	8.22	9.86
Volume of MeOH (in mL)	2.07	4.16	6.23	8.31	10.39	12.46
MeOH/triglyceride molar ratio	(3:1)	(6:1)	(9:1)	(12:1)	(15:1)	(18:1)

Table.5 Variation in %KOH used as transmethylation catalyst

UPO weight (in g)	20	20	20	20	20	20
Triglyceride mass (in g)	17.78	17.78	17.78	17.78	17.78	17.78
KOH weight (in g)	0.09	0.18	0.27	0.36	0.44	0.53
% KOH	0.51	1.01	1.52	2.02	2.47	2.98

Table.6 Variation in reaction medium temperature during transmethylation

Order numbers	1	2	3	4	5
Temperature of reaction medium (°C)	42	50	55	60	65

Table.7 Physicochemical characteristics of UPO

Measured parameters	Mean values \pm standard deviations
Water and volatile matter content (%)	0.15 \pm 0.01
Volumic mass at 20°C (g/mL)	0.9005 \pm 0.0007
Refractive index at 20°C	1.4653 \pm 0.0006
Acid value (mg de KOH/g of oil)	20.22 \pm 0.06
Saponification value (mg de KOH/g of oil)	182.30 \pm 0.01
Percentage of free fatty acids (%FFA)	9.23 \pm 0.11
Ester percentage (%)	88.91 \pm 0.05
Molar mass (g/mol)	1038.39 \pm 0.25

Table.8 RI and Vm of biodiesel produced in comparison with UPO

Measured parameters	UPO	Biodiesel
RI	1.4653 \pm 0.0006	1.4495 \pm 0.0007
Vm (g/mL)	0.9005 \pm 0.0007	0.8595 \pm 0.0007

Fig.1 Variation in FFA content according to the MeOH/FFA ratio

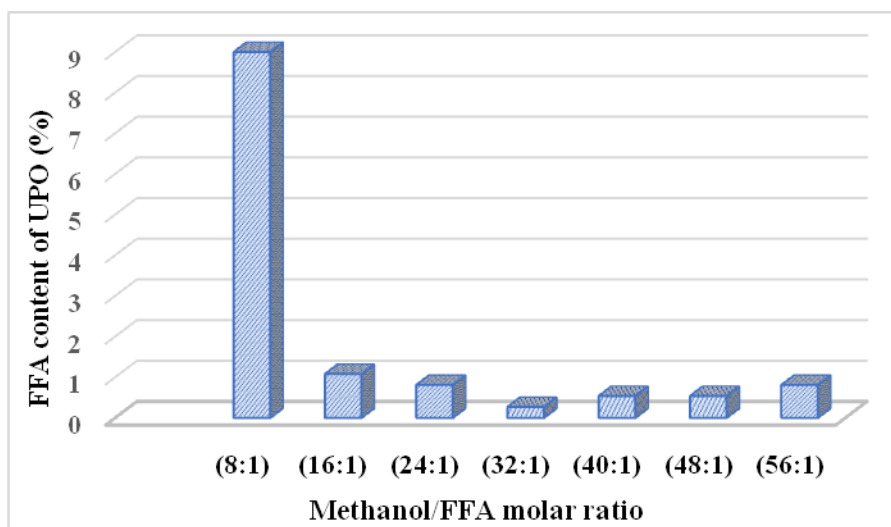


Fig.2 FFA content depending on catalyst content

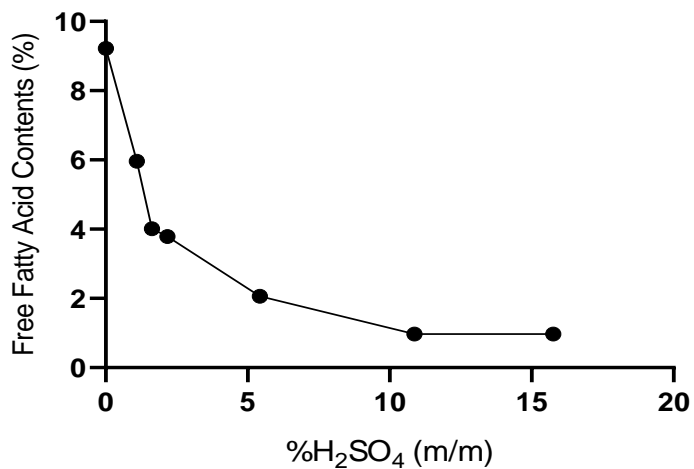


Fig.3 FFA content variation along time

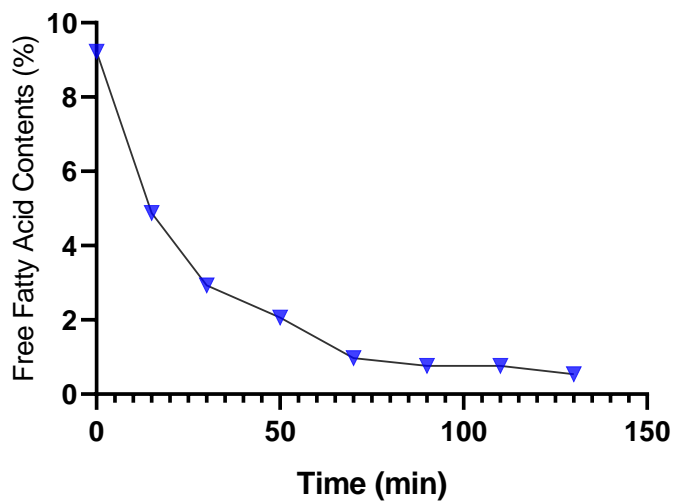


Fig.4 Evolution of RI of FAMES formed basing on %KOH

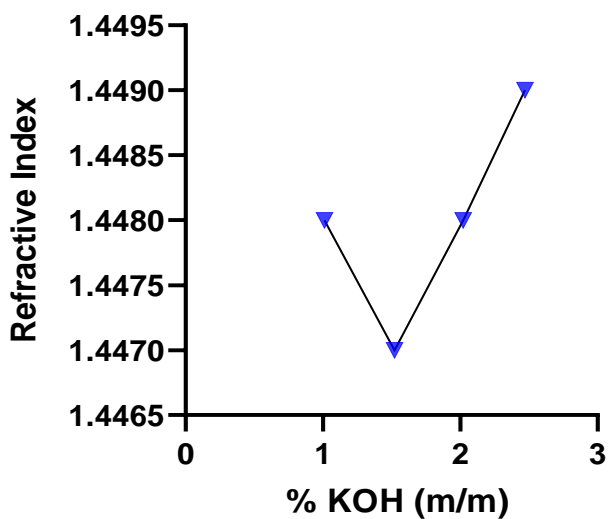


Fig.5 RI variation with reaction medium temperature

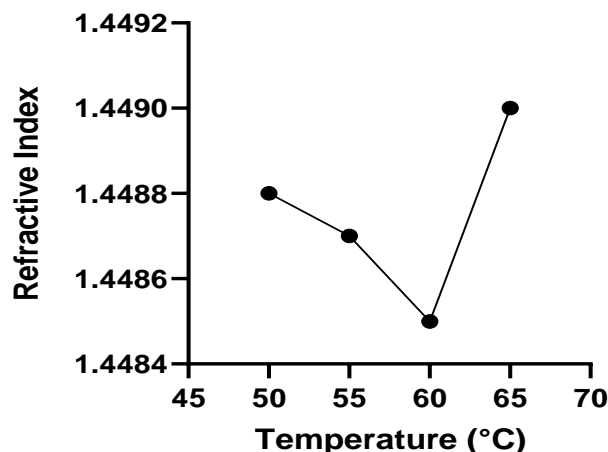
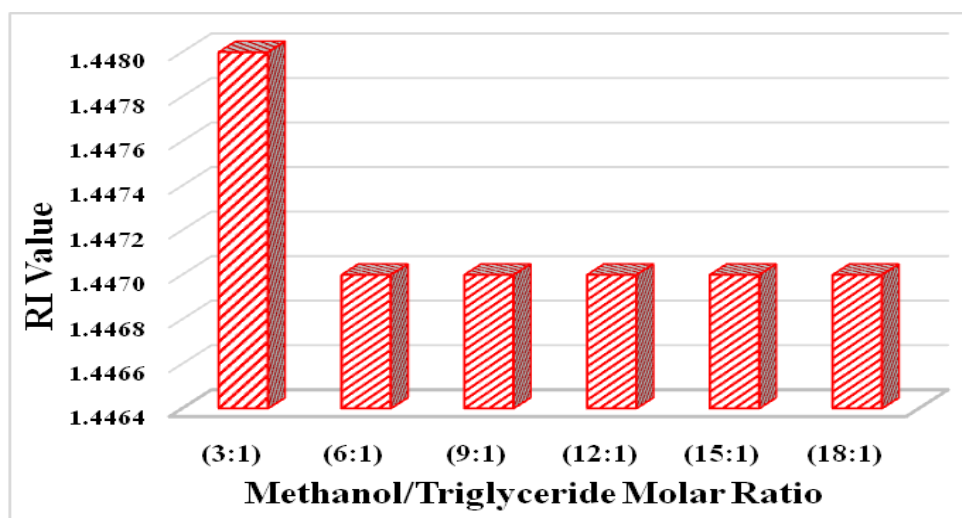


Fig.6 Evolution of RI according to the methanol/triglyceride molar ratio



Catalyst content influence on the FFA esterification reaction

The result obtained on FFA esterification reaction by using H_2SO_4 as catalyst in the current study was similar to the work already carried out by Canakci and Van Gerpen (2001), who had reduced the FFA content in fats with a high free fatty acid content from 98.69% using 25% H_2SO_4 as catalyst. The esterification reaction had progressed until it reached an equilibrium state, when the FFA content had reached the value of 0.97% to 10.87% H_2SO_4 , due to the accumulation of water in the reaction medium, resulting in the reverse reaction, well known as ester hydrolysis. In fact, the accumulation of water leads to a reduction phenomenon in catalytic activity of

H_2SO_4 , as the latter ends up in the aqueous phase (Canakci and Gerpen, 2001).

Effect of time on the esterification of FFA from UPO

The esterification time of FFA contained in UPO by acid catalyst with H_2SO_4 confirmed the work carried out by Encinar *et al.*, (2011). Indeed, these authors had observed that under conditions such as: (15:1) for the alcohol/oil molar ratio, 65°C for the temperature of the reaction medium, and 0.5% for the catalyst ratio (m/m oil), FFA conversion to their methyl esters as a function of time had allowed reduction of 98.9% after 180 minutes of reaction.

Transmethylation reaction optimization

Influence of KOH content on the transesterification reaction

It was 1.52% of KOH that would have provided the best biodiesel, characterized by the lowest refractive index of 1.4470. Indeed, the substitution of glycerol, a fatty molecule, by methanol rendered the FAMES formed in less refractive. As a result, the refractive index of the biodiesel produced would be considerably reduced. The optimal %KOH value found in the current study was not so far from that reported in the literature by Hayyan *et al.*, (2011), *i.e.*, 1% of KOH.

Influence of temperature on transesterification reaction

The result of optimal temperature about the transesterification reaction of UPO was in agreement with the work carried out by Gerpen *et al.*, (2004). These authors found that the use of alkaline bases (NaOH and KOH) as catalysts at 60°C optimized the transesterification reaction.

Specifically, the increase in the RI value observed in this study at a reaction medium temperature of 65°C could be due to the fact that the reaction medium temperature was close to the boiling point of methanol, *i.e.*, 64.7°C, thus inducing less contact between glycerides and methanol, because of the methanol volatilization phenomenon. Consequently, there was a drop in reactivity.

Influence of methanol/triglyceride molar ratio on transesterification

The methanol/oil ratio value revealed in the current study was not far from that obtained by Bojan and Durairaj (2012), *i.e.* (5.41:1). On the other hand, a large proportion of methanol corresponding to the methanol/oil ratio (10:1) had been reported by (Hayyan *et al.*, 2011) during transmethylation by basic catalysis with KOH. Similarly, Lapuerta *et al.*, (2008) had obtained a methanol/oil ratio equivalent to (9.2:1) with 0.5% of KOH.

Refractive index and volumic mass of biodiesel of UPO

The values of RI and Vm of biodiesel obtained from UPO were well lower than those corresponding to UPO,

used in this study as feedstock, *i.e.*, 1.4653 ± 0.0006 and 0.9005 ± 0.0007 g/mL, respectively.

This shows that the biodiesel produced had different characteristics compared to the starting oil used as feedstock. The reason for this would be that the substitution of glycerol by methanol had made the biodiesel less viscous, therefore it would be better suited to replace diesel.

With global energy crises constantly rocking the world, people decide to look for renewable energies. Among the sustainable options, biofuels offer excellent perspectives. Although biofuels are seen as clean energy sources able to replace fossil fuels, their expansion can unfortunately compete with food resources.

This is why the transformation of inedible oils into biodiesel appears to be a better alternative for the substitution of diesel. Consequently, the use of UPO in the current work presented a twofold challenge: reducing GHGs emissions and fighting against environmental pollution by non-edible oils.

This study showed that to convert UPO into biodiesel by using basic catalysis in a homogeneous phase, the feedstock would first have to be pre-treated, as the FFA content had exceeded the recommended threshold. The optimum pretreatment conditions were: molar ratio MeOH/AGLs = 24/1, %H₂SO₄ = 10.27 and reaction time = 90 min, while for the oil transmethylation reaction they were: molar ratio MeOH/triglyceride = 6/1, %KOH = 1.51 and reaction medium temperature = 60°C. Taking these optimum conditions into account, a biodiesel with interesting properties was obtained, thanks to its lower viscosity than the initial oil. This biodiesel could be an excellent alternative to diesel.

However, for its possible use in diesel engines, it would be canny to carry out preliminary tests on these types of engines first.

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Conflict of interest

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

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