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Contribution to the preparation of biofuels from some agricultural and industrial waste the case of Ouargla Region

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وزارة التعليم العالي والبحث العلمي
جامعة قاصدي مرباح - ورقلة

رقم
الترتيب:.............

كلية الرياضيات وعلوم المادة
قسم الكيمياء

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تحت عنوان:

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Dedication

I dedicate this work to my dear parents: my father Abdelhafid and my mother Oumelkheir who have supported me from my birth until today.

I will never know how to thank you for their encouragement and advice during the period of my studies, can not express love, esteem, the dedication and respect that I have always seen.

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To my brother wife: Bouchra and to my dear: Lina.

To all my big family: BEN CHEIKH and BETTAYEB

To my friends: Kamilia, Sabah, Nadjah, Heba, Soumia, Hanane, Tatou, Wafa, chaima, Zaineb, Ahlem, Saida, Asma

At the end I dedicate it to the people who have contributed from near or far to the development of this work

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Abstract

The demand for fossil fuels is increasing day by day to meet human needs and daily activities, but the latter has a negative impact on the environment and human health due to the emissions of toxic gases resulting from its combustion which affect the global warming, which made the world think of a new economic source, less harmful and more respectful of the environment.

This study aims to recover some waste and materials of low value or harmful to the environment and to transform them into high quality green; "bio" fuels. The results of the study showed that can be valued the waste cooking oil to produce biodiesel by transesterification reaction using three (03) different methods: ultrasonic transesterification, which gave the highest yield of 99.7%, using sodium hydroxide as a catalyst. The second, using microwave method, with a yield of 94.4%, but the lowest yield was 79% using the conventional esterification method.

The results of improving the yield using the microwave method have shown that the optimum conditions for the best yield are: a catalyst concentration of 1% (by weight), a molar ratio of 1: 9 methanol: oil, 8 minute as reaction time at 70°C and 800 rpm. The results of this work also showed that we can use the date variant "Degla Bayda" as a low cost feedstock to produce bioethanol by fermentation with the commercial yeast *Saccharomyces cerevisiae*, which gave the best yield of 36.25 % under optimal conditions represented by: a temperature of 32°C, pH of 5, amount of yeast 1 g and fermentation time of 48 hours (two days).

The physico-chemical properties of the biofuel produced were determined and compared to American standards (ASTM) and European standards (EN).

Keywords: Waste cooking oil, transesterification, biodiesel, dates, fermentation, bioethanol.

Résumé

La demande d'énergie fossile augmente de jour en jour pour répondre aux besoins humains et aux activités quotidiennes, mais cette dernière a un impact négatif sur l'environnement et la santé humaine en raison des émissions de gaz toxiques résultant de sa combustion qui affectent le réchauffement climatique, ce qui a fait le monde pense à une nouvelle source économique, moins nocive et plus respectueuse de l'environnement.

Cette étude vise à valoriser certains déchets et matières premières de faible valeur ou nocifs pour l'environnement et à les transformer en carburants verts "bio" de haute qualité. Les résultats de l'étude ont montré que l'huile de cuisson usagée peut être valoriser pour produire du biodiesel par réaction de transestérification selon trois (03) méthodes différentes : la transestérification par ultrasons, qui a donné le rendement le plus élevé de 99,7 %, en utilisant de l'hydroxyde de sodium comme catalyseur. La seconde méthode, utilisant les micro-ondes, avec un rendement de 94,4%, mais le rendement le plus bas était de 79% en utilisant la méthode d'estérification conventionnelle.

Les résultats d'amélioration du rendement en utilisant les micro-ondes méthode ont montré que les conditions optimales pour le meilleur rendement sont : une concentration de catalyseur de 1% (en poids), un rapport molaire de 1:9 méthanol: huile, un temps de réaction de 8 minutes à 70°C et 800 tr/min. Les résultats de ce travail ont également montré que nous pouvons utiliser la variante de dattes "Degla Bayda" comme matière première à faible coût pour produire du bio-éthanol par fermentation avec la levure commerciale *Saccharomyces cerevisiae,* qui a donné le meilleur rendement de 36,25% dans des conditions optimales représentées par : une température de 32°C, un pH de 5, une quantité de levure de 1 g et un temps de fermentation de 48 heures (deux jours).

Les propriétés physico-chimiques du biocarburant produit ont été déterminées et comparées aux normes américaines (ASTM) et européennes (EN).

Mots clés : Huiles de cuisson usagées, transestérification, biodiesel, datte, fermentation, bio-éthanol.

الملخص

يزداد الطلب على طاقة الوقود الاحفوري يوما بعد يوم لتلبية متطلبات الانسان وأنشطته اليومية، إلا أن هذا الأخير له تأثير سلبي على البيئة وصحة الأنسان وذلك بسبب انبعاثات الغازات السامة الناتجة عن احتراقه والتي تؤثر في الاحتباس الحراري وهو ما جعل العالم يفكر في مصدر اقتصادي جديد أقل ضررا وأكثر صداقة للبيئة. تهدف هذه الدراسة إلى تثمين بعض المخلفات والمواد الأولية ذات القيمة المنخفضة أو الضارة بالبيئة وتحويلها إلى وقود أخضر (حيوي) عالي الجودة. أظهرت نتائج الدراسة أنه يمكن تثمين زيت الطهي المستعمل لإنتاج الديزل الحيوي عن طريق تفاعل الأسترة التبادلية بثلاث طرق مختلفة متمثلة في الأسترة باستخدام طريقة الموجات فوق الصوتية والتي أعطت أعلى مردود بنسبة 99,7% وذلك باستخدام هيدروكسيد الصوديوم كمحفز، أما بالنسبة للطريقة الثانية فباستعمال طريقة الموجات الميكروية والتي أعطت مردود بنسبة 94.4%، إلا أن أقل مردود بلغ 79% باستعمال طريقة الأسترة العادية. وأبرزت نتائج تحسين المردود في هذه الدراسة باستخدام الطريقة الميكروية أن الظروف المثلى لأفضل مردود هي 1% (بالوزن) لتركيز المحفز، 1:09 نسبة المولارية ميثانول: زيت، زمن التفاعل 8 دقائق عند 70 درجة مئوية، و800 دورة في الدقيقة. كما أظهرت نتائج هذا البحث أيضا أنه يمكننا استخدام تمر "الدقلة البيضاء" كمادة منخفضة التكلفة لإنتاج الإيثانول الحيوي عن طريق التخمر وذلك بالخميرة التجارية *Saccharomyces cerevisiae* حيث أعطت أحسن مردود بنسبة 36.25% في ظروف مثلى متمثلة في :درجة الحرارة 32 درجة مئوية، ودرجة الحموضة 5، وكمية الخميرة 1 غ وزمن التخمر لمدة 48 ساعة (يومين).

تم تحديد الخصائص الفيزيائية والكيميائية للوقود الحيوي المنتج ومقارنتها بالمعايير الأمريكية (ASTM) والمعايير الأوربية (EN).

الكلمات المفتاحية: مخلفات زيت الطهي، الأسترة التحويلية، الديزل الحيوي، التمر، التخمر، الإيثانول الحيوي.

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CHAPTER.I: Bibliography of biofuel

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List of abbreviations

List of Abreviations

ADH	Alcohol Dehydrogenase
ASTM	American Society for Testing and Materials
AN	Acid Number
CN	Cetane Number
CV	Conventional
CP	Cloud Point
Cat.	Catalyst
DG	Diglycerides
DNS	3,5-Dinitrosalicylic Acid
Eq.	Equation
EU	European Union
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FT-IR	Fourier Transform Infrared spectrometer
FP	Flash Point
GL	Glycerol
GC-MS	Gas Chromatography coupled with a Mass Spectrometer
HPLC	High-Performance Liquid Chromatography
IC	Internal Combustion
ISO	International Organization for Standardization
IV	Iodine Number
IR	Infrared spectroscopy
LC-RID	Liquid Chromatography-Refractive Index Detector
MG	Monoglycerides
MeOH-oil	Methanol/oil
MW	Microwave-assisted
MeOH	Methanol
P	Power
PP	Pour Point
Q	Quantity
SN	Saponification Number
S. cerevisiae	*Saccharomyces cerevisiae*
TG	Triglycerides
T	Temperature
t	Time
UT	Ultrasound-assisted
UV–vis	UV–visible spectrophotometry
USA	United States of America
VOME	Vegetable Oil Methyl Ester

VOEE	Vegetable Oil Ethyl Ester
v/v	Volume/ volume
wt.	Weight
w/v	Weight /volume
WCO	Waste Cooking Oil
Y	Yield

Summary

Summary

CHAPTER.I: Bibliography of biofuel

CHAPTER II: Biodiesel preparation methods

CHAPTER III: Bioethanol preparation methods

General Introduction

Energy demand keeps increasing day by day the most basic requirement for human survival and activities is due to the increase in population, industrialization, and fast modernization. fuel is an important backbone of the world's economy [1,2]. Fossil fuels perform an important role in the development of industrial growth, transportation purpose, and agricultural area, and also satisfy other basic needs [3]. In the present world, fossil fuels availability decreasing drastically, which increases the price of fossil fuels [2].

Apart from this, increasing energy use, climate change, and carbon dioxide (CO_2) emissions from fossil fuels make switching to low-carbon fuels a high priority [4]. Therefore, the future energy demand is moving toward renewable and environmentally friendly, and sustainable energy sources. Different renewable energy sources, like solar energy, geothermal energy, wind energy, energy from biomass…. etc, can be used as alternative energy sources in place of fossil fuels. Among these renewable energy sources, bioenergy in general and biofuels (biogas, bio-alcohols, and biodiesels) in particular, which are fuels obtained from biomass [2,5,6].

Biodiesel is an alternative liquid fuel that can substantially replace conventional diesel and reduce exhaust pollution and engine maintenance costs. This renewable fuel can be produced from different feedstock such as (edible, non-edible oils including animal fats) [4,7]. Biodiesel can be produced with various methods from a wide range of feedstocks [8]. The methods include transesterification [9], direct blending [10], pyrolysis [11] and micro-emulsion [12]. Generally, biodiesel is derived from triglycerides by transesterification with alcohol preferably methanol in the presence of a catalyst (alkali-catalyzed, acid-catalyzed, enzymatic, supercritical) [7].

Bioethanol is ethanol extracted by fermentation. The latter is anaerobic bioconversion in the presence of yeast and inappropriate conditions of temperature and pH, the fermentation of sugars contained in the biomass [13]. Vegetable biomass as feedstock for bioethanol production allows for recycling the CO_2 released during combustion, reducing the CO_2 emissions [14]. Nowadays, the commercial bioethanol is almost entirely of first generation since food crops are used as feedstock: sugarcane in Brazil, corn in the US, and wheat and sugar beet in the European Union (EU), rice straw [15], tea processing waste [16,17], rice hull [18,19], barley husk, wheat bran, rye bran [20], Ulva Lactuca [21], and taro waste, and the production of ethanol is increasing every day [22,23]. The main disadvantage of first generation bioethanol is the competition over of arable land for the cultivation of food crops between biofuel feedstocks, thus increasing food prices [14,24].

The accumulation of a large amount of waste and bad dates that are not suitable for consumption it negatively affects the environment and human health and this is makes us think of a way to exploit and benefit from them in order to preserve the environment and create jobs.

In this research, we aim to evaluate some waste and materials of low value or harmful to the environment into green (bio) fuels of high quality with the modern method. For this purpose, we use waste cooking oil (WCO) to produce biodiesel because it depleted it in nature and so returned these worthless materials to the consumption cycle via green fuel production. we used also "Degla Bayda" dates biomass to produce biodiesel because it is a product with market value (poor quality) in Algeria. Finally, we checked the quality of the two producers by analyzing their physicochemical properties and comparing them with international standards.

This thesis is subdivided into three chapters:

- **The First Chapter:** Bibliography of biofuels
- **The Second Chapter:** Biodiesel preparation methods
- **The Third Chapter:** Bioethanol preparation methods

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Chapter I

Bibliography of biofuels

I.1. Introduction

Renewable energy also called green energy, is derived from natural resources that is renewable and does not run out. It is environmentally friendly energy because the emissions resulting from it are carbon dioxide (CO_2) or (NO_2, SO_2) that increase global warming are few compared to traditional non-renewable energy [1,2]. Renewable energy sources vary such as solar energy, wind energy, hydro energy, and bioenergy [3]. In this chapter, we will tackle one of the most important clean energies and its effects on the environment, which is bioenergy, represented in biofuels.

I.2. Biofuel

Biofuels are energy sources derived from biological materials that distinguish them from other non-fossil energy sources, such as wave energy. A biofuel (or agrofuel) is a fuel produced from non-fossil organic materials, derived from biomass [4].

There are currently three types [5]:

- ✓ **Biodiesel:** (vegetable oil esters), oil and derivatives sector
- ✓ **Bio-alcohol:** of sweet juice, starch, cellulose, or hydrolyzed hemicelluloses
- ✓ **Biogas:** resulting from the fermentation of organic waste away from the air.

I.2.1. Different generations of biofuels

I.2.1.1. First-generation biofuels

First generation biofuels are mainly divided into two sectors, according to the two main types of internal combustion engines:

a. The oil sector:

This sector is mainly based on rapeseed, but sunflower, coconut oil, or palm oil can also be raw materials for this sector. Outside of agriculture, the oil sector also has great potential through the recycling of used vegetable and animal oils.

b. The sugar sector:

Mainly use sugar beet and wheat as raw materials, and could also use potato. In other regions, ethanol is produced from soybeans (USA) or sugar cane (Brazil). In this sector, it is the use of cellulosic material, waste paper, wood and wood waste (recycling of pallets, sawmill residues, etc.), straw, energy crops (short rotation coppice) that has the greatest potential for low-cost production [6]

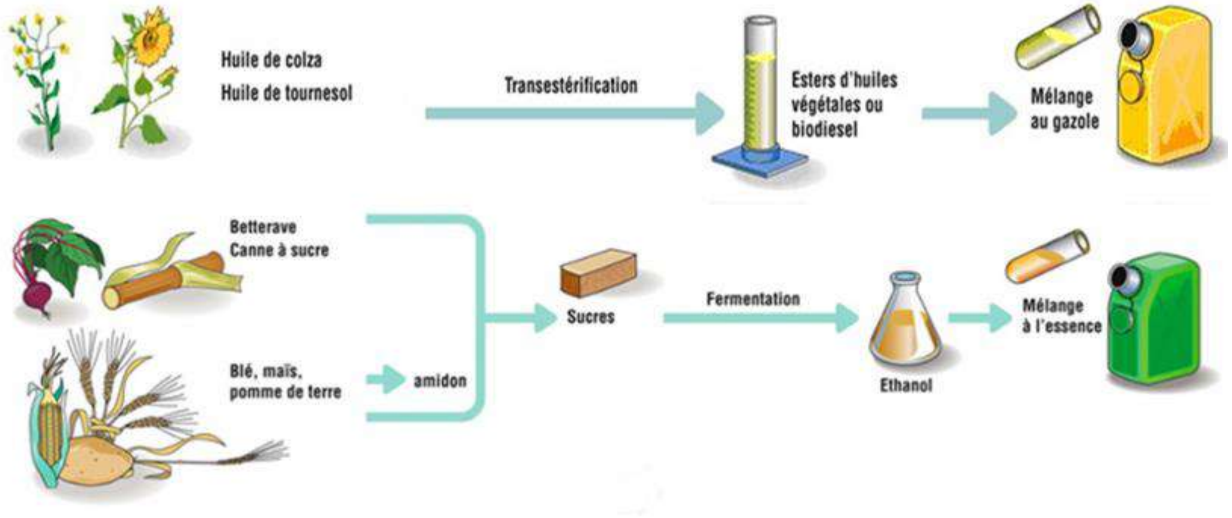


Figure I.1: Phases of first-generation biofuel production

I.2.1.2. Second-generation biofuels

A second-generation biofuel is obtained from biomass dedicated to non-food, less demanding in terms of the quality of arable land and water. It is more specifically the lignocellulosic biomass (lignin and cellulose) [7]. associated with inedible parts of plants, waste, agricultural crop residues, forestry activities, as well as the organic fraction. It then becomes possible to develop straws, stems, leaves, or even fast-growing plants (*Miscanthus* and *Jatropha curcas*) whose oil is inedible and which arouses general enthusiasm on the part of the scientific community because of its significant potential for the production of biodiesel [6,8].

The fundamental advantage of second-generation biofuels is that they reduce the problematic duality between food production and biofuel production, allowing for more efficient use of cropland.

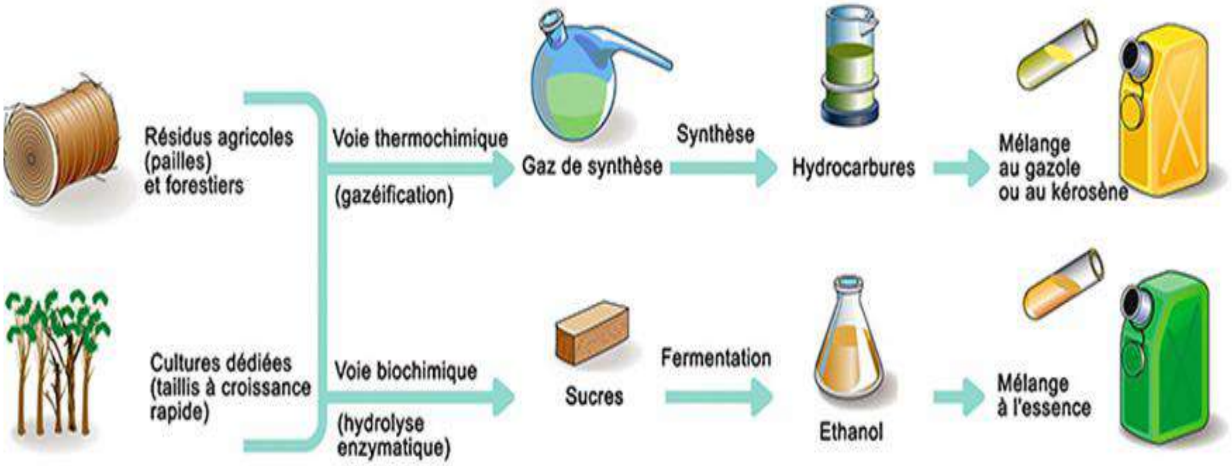


Figure I.2: Phases of second-generation biofuel production

I.2.1.3. Third-generation biofuels

These are agrofuels from microalgae or algo-fuels. Microalgae can provide different types of renewable energy. These include methane produced by the anaerobic digestion of algae, biodiesel derived from the oil of microalgae.

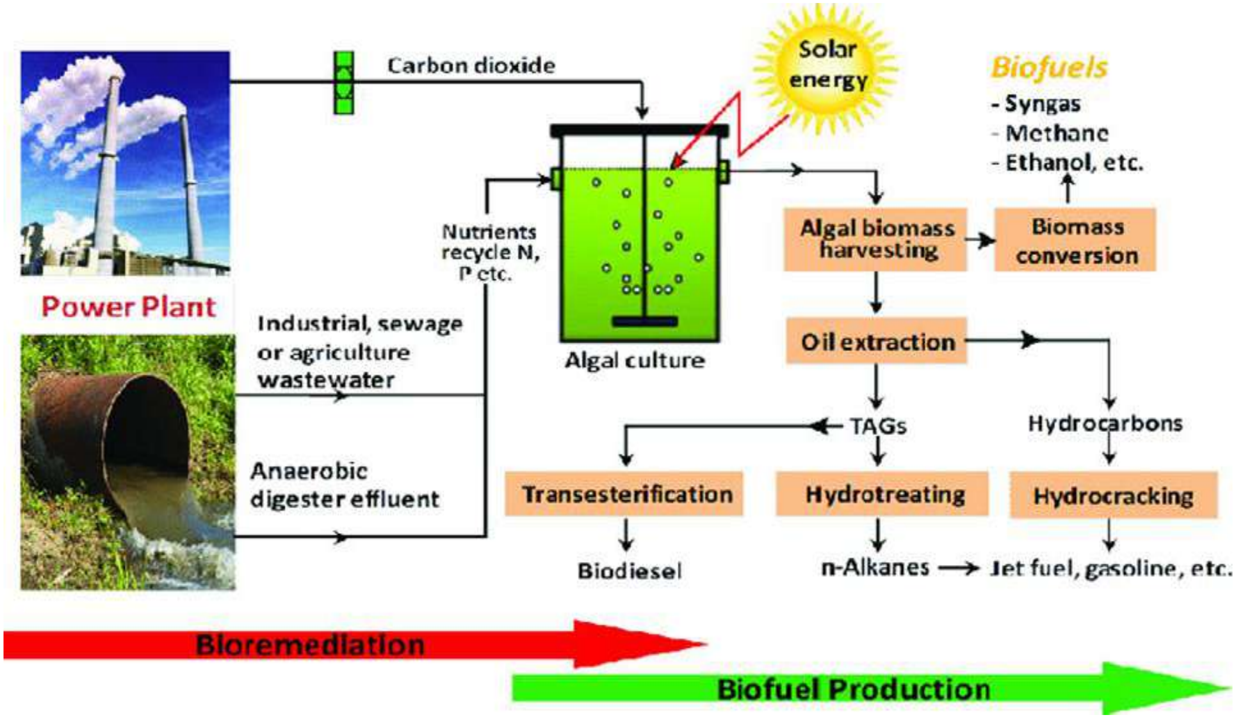


Figure I.3: Phases of third-generation biofuel production [9]

Depending on the type of biomass, there are two major methods of its conversion: the first is biotechnological conversion and the second involves thermal conversion. The following diagram (Fig. I.4) shows the methods of converting the biomass to biofuel:

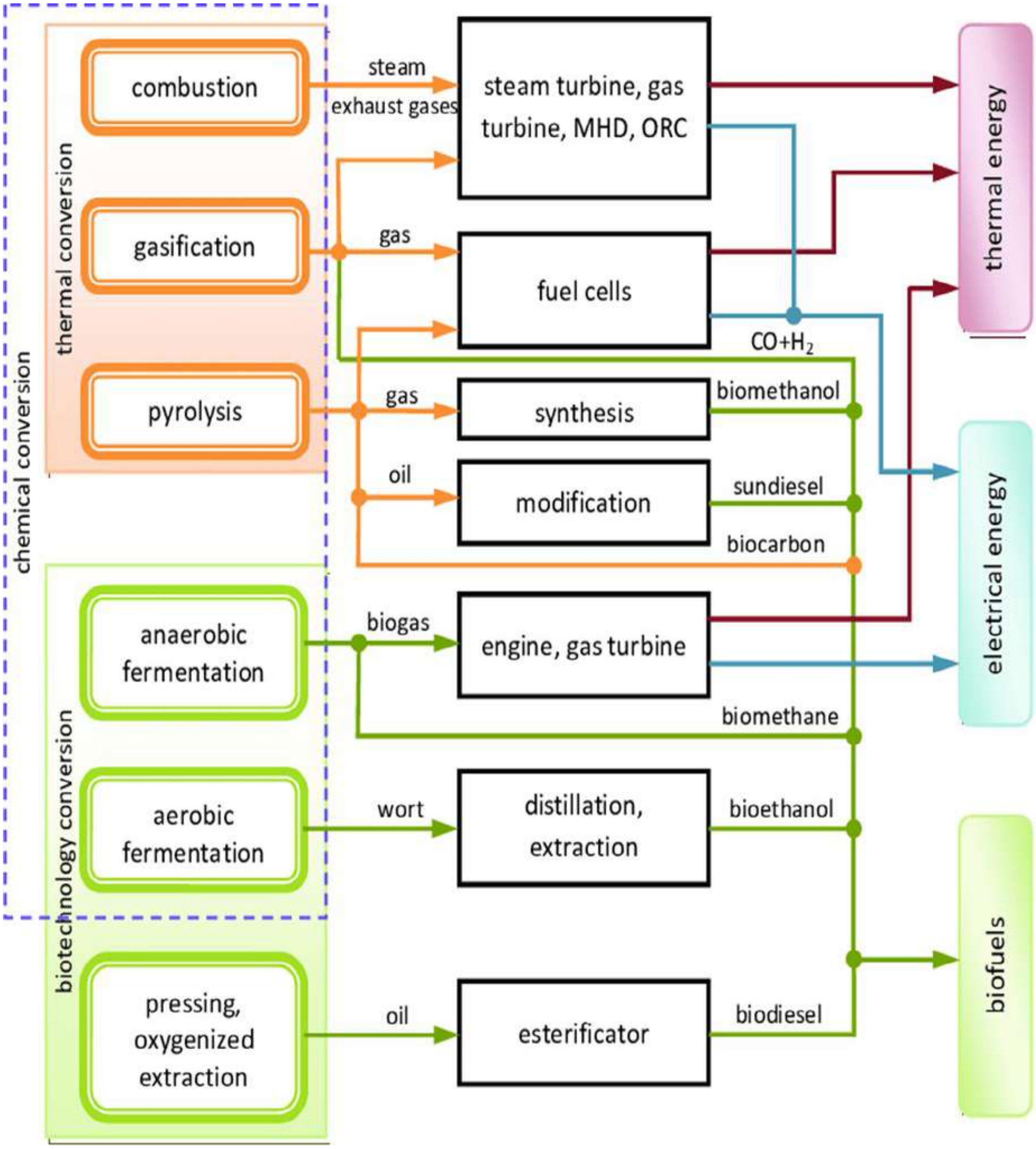


Figure I.4: Methods of energy production from biomass [10]

I.2.2. The advantages and disadvantages of biofuels

I.2.2.1. Advantages of biofuels

The benefits of using biodiesel in internal combustion (IC) engines are profound and are marked for its wider applications based on the following highlights [11,12]:

- **Cost:** Biofuels are much less expensive than gasoline and fossil fuels.
- **Resource:** Fossil fuels are a limited resource (it takes thousands of years to produce), while biofuels can be produced from several biological and renewable materials.
- **Renewables:** It takes thousands of years to produce fossil fuels. while the feedstock for biofuels is organic and easy to source. More biodiesel products can be made available from different plant oil materials and waste oil.
- **Biodegradability:** Biofuels are easily biodegradable and much less dangerous to handle than traditional fuels.
- **Low Emission:** biodiesel is a clean-burning fuel. The studies suggest that the combustion of biodiesel reduces GHG by 40%–65%. It is a safer alternative to preserve atmospheric quality.
- **Security:** biodiesel is non-toxic and also safe to transport from one point to another owing to its relatively high flash point.
- **Economic stimulation:** Provision of foreign exchange-earners because biofuels are produced locally, biofuel manufacturing plants can create new jobs in rural areas. The production of biofuels will also increase the demand for biofuel crops, thereby boosting the economy of the agricultural industry.

I.2.2.2. Disadvantages of biofuels

It is important to keep in mind that despite the advantages that characterize the use of biofuels, their production and end-use may have serious environmental [12]**:**

- **Energy production**: Biofuels are less energy efficient compared to conventional fuels, and therefore require the largest amount to produce the same level of energy.

- **High Cost:** Refining biofuels to produce more efficient energy and building the manufacturing plants needed to increase quantities of biofuels will require large initial investments.
- **Water use:** huge amounts of water are required for good irrigation of biofuel crops, which may strain water resources at the local and regional levels.
- **Availability:** Biofuels are not yet readily available to consumers and most vehicles are not equipped to run on biofuels.

I.2.3. Effects of biofuels on the environment

A study indicates that biofuels have a positive impact on the environment in contrast to fossil fuels, whose combustion leads to the production of a high percentage (72%) of carbon dioxide and greenhouse gases CO_2, NO_2, SO_2), unlike biofuel, which reduces emissions The greenhouse gases by (65%- 40%) [11,13]. Because the combustion of both types of fuel does not have the same effect as shown in figure (I.5), where fossil fuels produce toxic carbon dioxide in the atmosphere, which leads to global warming, unlike biofuels, which burn better because it contains the oxygen atom is in a chemical formula, where the carbon emitted from it acts as a source of energy for plants (they use it in the photosynthesis process). As a result, biofuels are more environmentally friendly[2] [14].

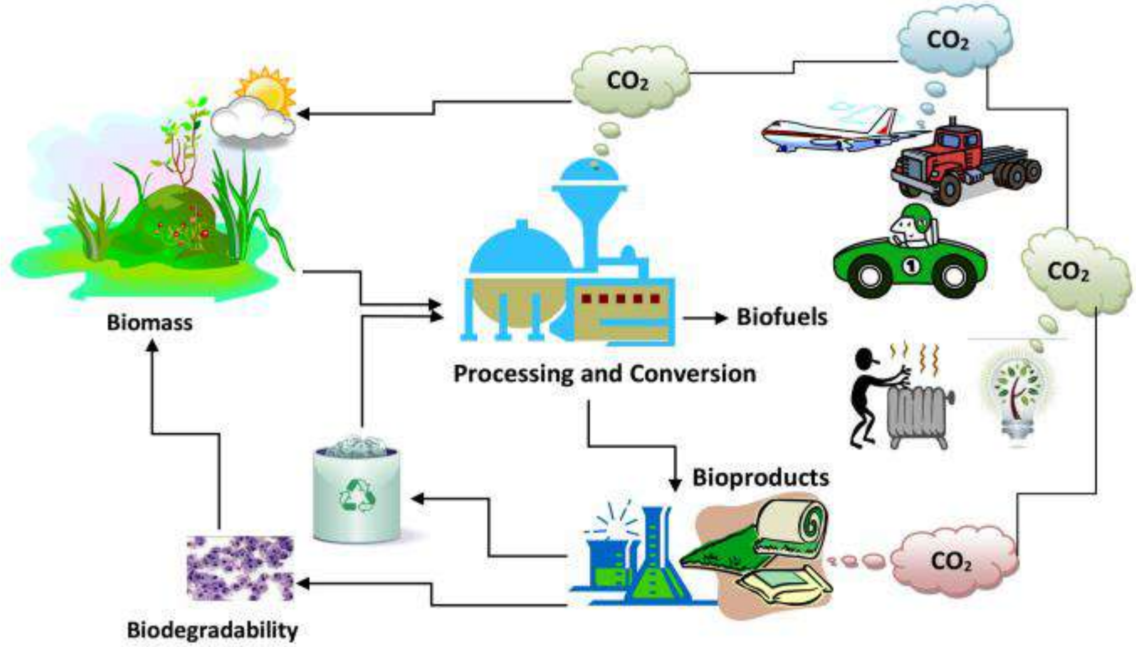


Figure I.5: Biomass life cycle for biofuels.

I.2.4. International production of biofuels

In recent decades, we have witnessed the emergence of liquid biofuels in contrast to with traditional fuels (gasoline and diesel). The demand for them is increasing in the global market as shown in the figure I.6 According to estimates, the global production of biofuels increased by a percentage from 2016 to 2019 [15]. The global production of biodiesel is concentrated on four producers, accounting for 78.63% of the total production. The European Union ranks first (31%), followed by the United States (19%), then Indonesia and Brazil (16% and 13%, respectively) [16]. As for bio-ethanol, the largest percentage of global production is occupied by the United States with 50% and Brazil with 27%, as shown in figure (I.7) [17].

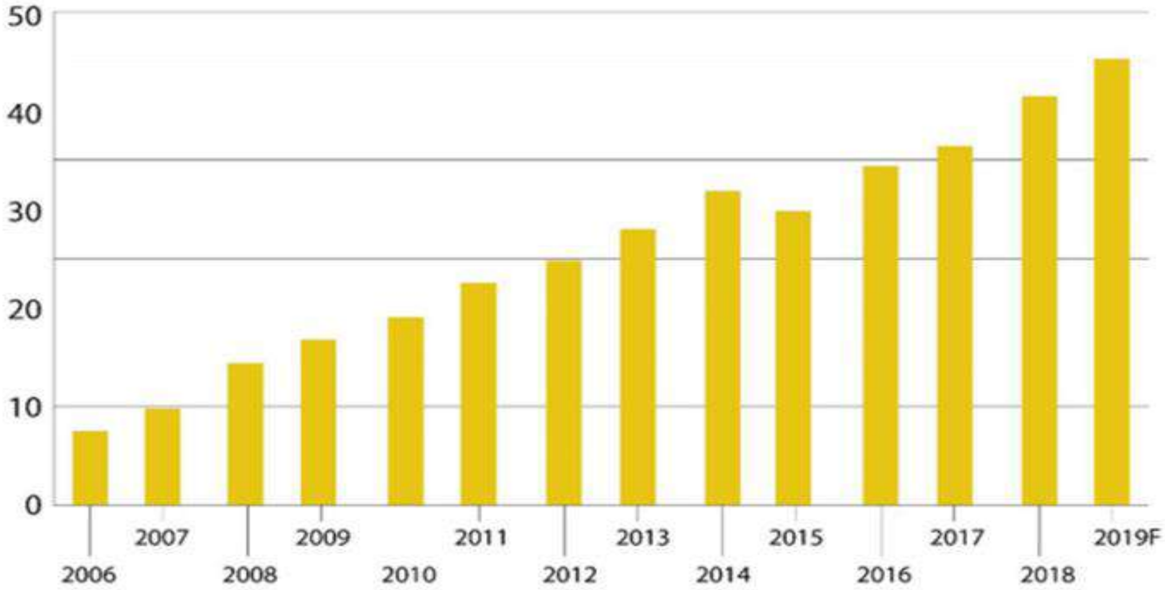
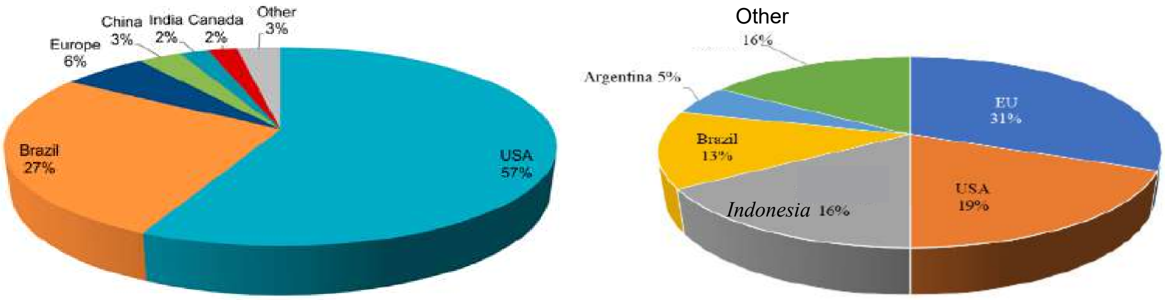


Figure I.6: World production of biodiesel (million tons).



A **B**

Figure I.7: The first countries in the production of biodiesel (A) and bioethanol (B)

I.3. Biodiesel

Biodiesel is a renewable, ecological, biodegradable liquid biofuel produced from renewable resources (vegetable oils, recycled frying oils, or animal fats) and which does not contain any petroleum products but can be used as diesel fuel in diesel engines [18].

In chemistry: Biodiesel is defined as the long-chain mono-alkyl ester of fatty acids derived from renewable lipids, such as vegetable oils and animal fats, for use as biodiesel. Table (I.1) shows the physicochemical properties of biodiesel [19].

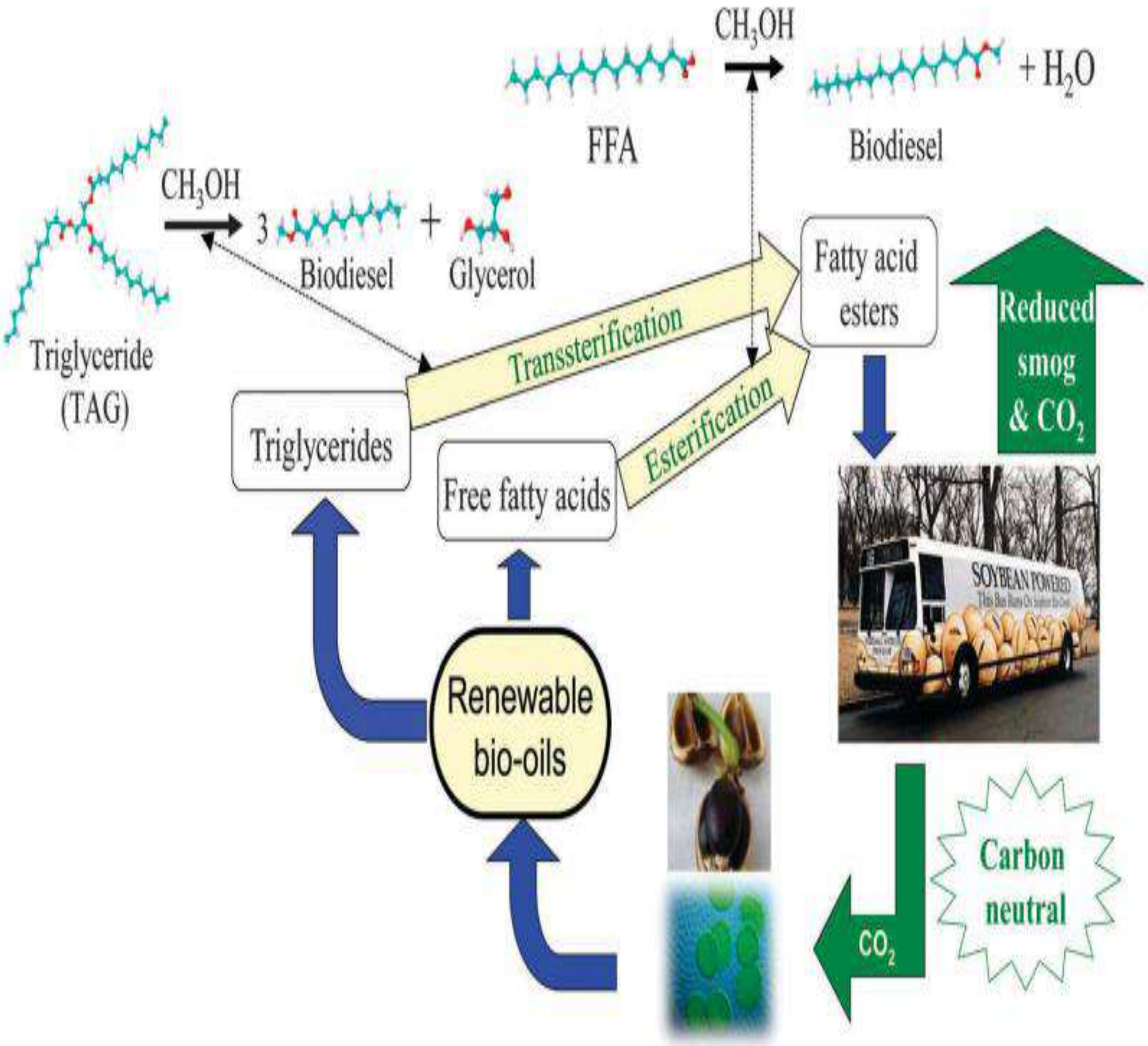


Figure I.8: Biodiesel production cycle from renewable bio-oils [6]

Table I.1: Physicochemical properties of biodiesel

Common name	**biodiesel**
Common chemical name	methyl or ethyl ester of fatty acid
Chemical formula	C_{14}–C_{24} methyl ester or $C_{15-25}H_{28-48}O_2$
Physical condition	Dark yellow liquid
Biodegradability	More biodegradable than diesel fuel
Solubility in water	Insoluble in water
Density (Kg/m^3, at 288K)	860–894
Kinematic viscosity (mm3/s, at 313K)	3.3–5.2
Boiling point (K)	>475
Flash point (K)	430–455
Distillation (K)	470–600

I.3.1. Biomass of biodiesel

The raw materials used in the production of biodiesel vary among countries, climates, and policies. But in general, we can classify these raw materials in three categories [20,21]:

- ✓ **Edible vegetable oils:** Soybean, colza, peanut, corn, coconut, rice, sesame, etc. But they are more expensive than other types of oil, which makes their use economically impractical.
- ✓ **Inedible vegetable oils:** as, such as jojoba, Jatropha, Karanja, neem (Azadirachta indica), microalgae. Hence the need to use inedible oilseed plants and trees that grow in uncultivated
- ✓ **Animal and vegetable fatty waste:** Animal greasy waste come either from restaurants such as vegetable oils and animal fats used for frying and cooking meat and fish or from slaughterhouses such as inedible fats remaining after carapace cutting and fish oil from the processing of fishery products.

I.3.2. Biodiesel production methods

To produce derivatives of vegetable oils having properties and performances quite similar to those of diesel. There are several ways to produce and use biodiesel, including:

I.3.2.1. Dilution

Dilution is the process of adding diesel fuel to triglycerides to thin them down (lower viscosity) for improved engine efficiency. This approach does not require any chemical processes. Because of variations in viscosity and pour points, it has been demonstrated that the total replacement of vegetable oils for diesel fuel is not experimentally feasible [22,23]. As a consequence, blending 20%–25% vegetable oil blends with diesel have been investigated to obtain quality performance outcomes for diesel engines [24,25]. Diluted fuels such as preheated palm oil and palm oil/diesel oil blends, as well as palm oil/waste cooking oil combinations, were created and effectively used as fuels in an IC engine [26].

I.3.2.2. Micro-emulsification

Micro-emulsification, also known as co-solvent blending, is the thermodynamic equilibrium dispersion of microstructures with an average diameter, d, smaller than 0.25 of the visible light wavelengths. It takes a lot of agitation to keep the combination in a single condition. A micro-emulsion can be created by dissolving immiscible compounds such as vegetable oils and an ester, or vegetable oils and alcohols such as methanol, ethanol, butanol, and hexanol in a co-solvent with the addition of a surfactant and a cetane improver. Depending on the engine type, the addition of diesel fuel is optional. Micro-emulsification is a reliable method for lowering triglyceride viscosity [28,29].

I.3.2.3. Pyrolysis

Pyrolysis is the anaerobic heat decomposition of organic molecules in the presence of a catalyst. Animal fats, vegetable oils, natural triglycerides, or FAME are examples of fragmented materials. Triglyceride pyrolysis commonly produces alkanes, alkenes, alkadienes, aromatics, and carboxylic acids [24,29]. The liquid components of disintegrating fats are similar to diesel fuels. The pyrolysis products have lower CN, viscosity, flash point, and pour point qualities than diesel fuel but equivalent heating values [27,30]

I.3.2.4. Transesterification

Transesterification is one of the most important methods used to convert vegetable oils into diesel fuel. It consists of esterifying the triglycerides of vegetable oils by adding alcohol, generally methanol or ethanol in the presence of a catalyst [21].

The transesterification reaction is generally the process of replacing the alkyl group of alcohol (R_2) with the alkyl group of an ester (R_1). The alcohols most commonly used in transesterification are methanol or ethanol, and methanol is used more commonly due to its abundance and low cost compared to other alcohols [3].

The transesterification reaction takes place in the presence of a catalyst and produces [31]:

- ✓ Vegetable oil methyl ester (VOME) when using methanol, or vegetable oil ethyl ester (VOEE) when using ethanol.
- ✓ Glycerol referred to as glycerin is a high-value by-product.

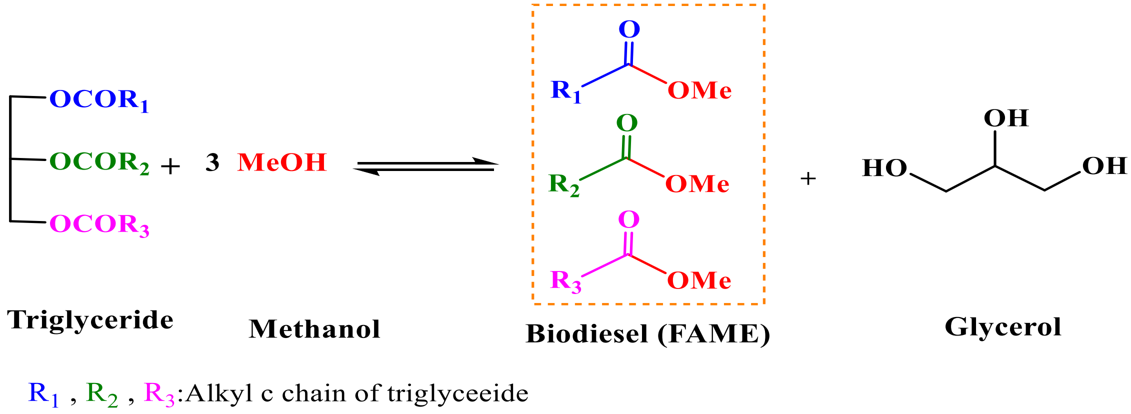


Figure I.9: General equation for the transesterification reaction

I.3.3. The mechanism of the transesterification reaction

The reaction mechanism of the esterification reaction is summarized in three consecutive and reversible steps: The first is the conversion of triglycerides (TG) to diglycerides. Then, diglycerides (DG) convert to monoglycerides (MG) which in turn convert to glycerol. (GL) At each step, a fatty acid ester molecule is formed.

I.3.3.1. The basic catalysts mechanism

The reaction mechanism of the esterification with the basic catalysts each of the previous transformations takes place in three steps, steps (1) and (2) fast because the ester functions are converted first, and step (3) is slower. As shown in figure (I.10) [20].

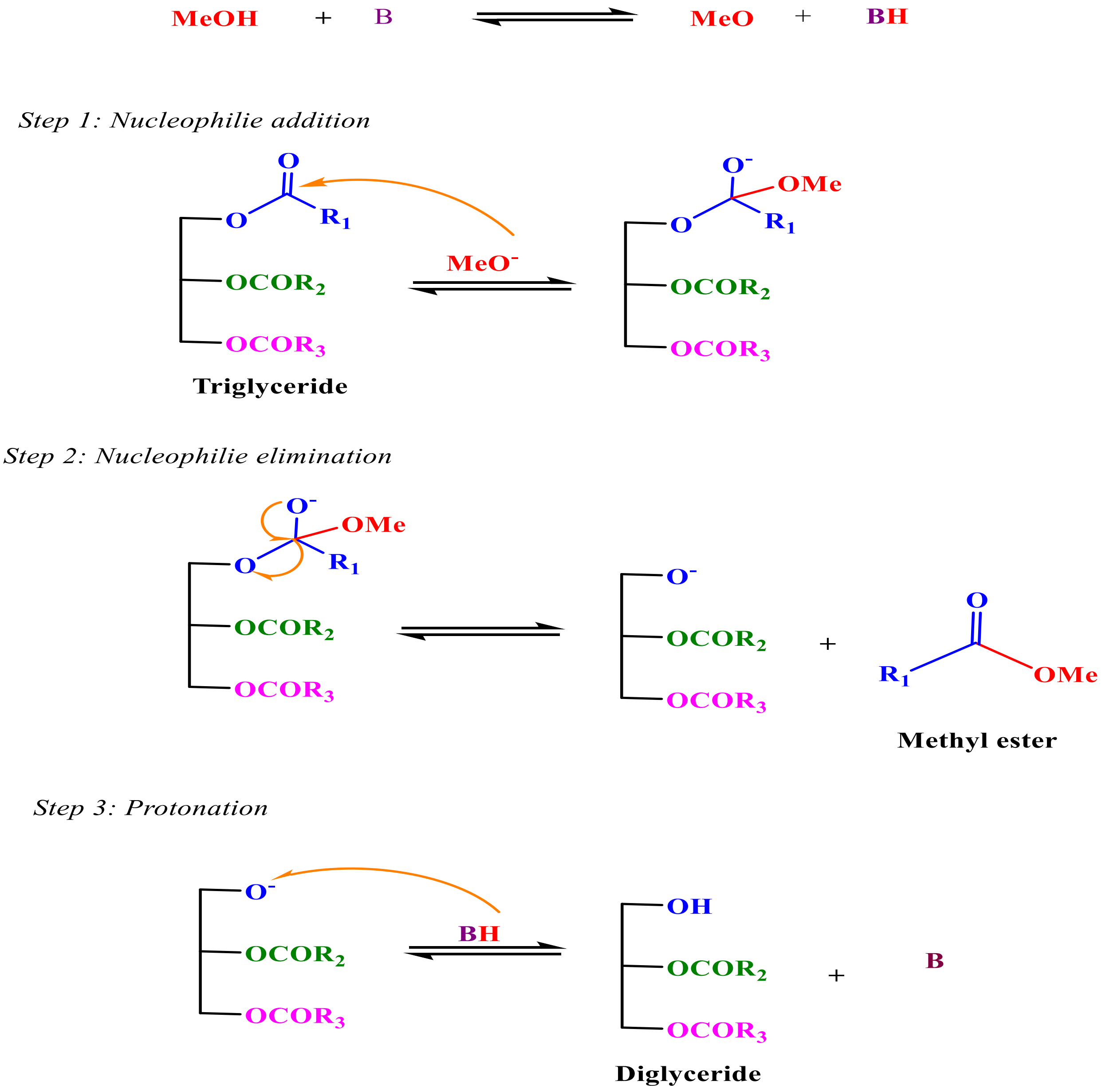


Figure I.10: Mechanism of the esterification reaction of triglycerides with methanol in the presence of a basic catalyst

I.3.3.2. Acid catalyst mechanism

The reaction mechanism of the esterification with the acid catalysts each of the three transformations takes place in four steps as shown in figure (I.11) [32].

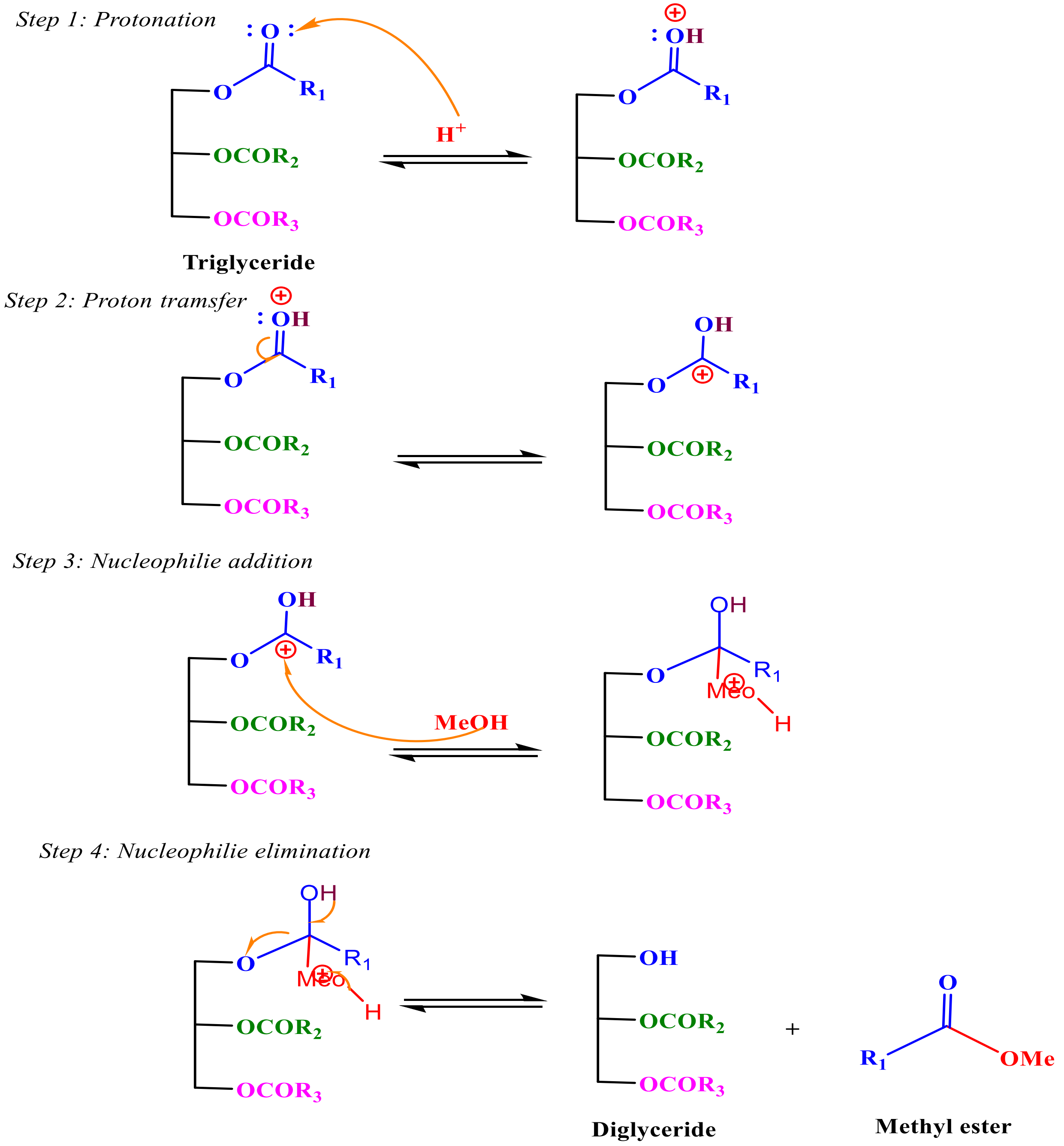


Figure I.11: Mechanism of esterification reaction of triglycerides with methanol in the presence of an acid catalyst

I.3.4. Effect of condition on the transesterification

I.3.4.1. Effect of rate and type of catalyst

Biodiesel is synthesized by transesterification with lower alcohols or by esterification of fatty acids in the presence or absence of a catalyst. there is a different type of catalyst used in the process of transesterification of oils for the production of biodiesel [33].

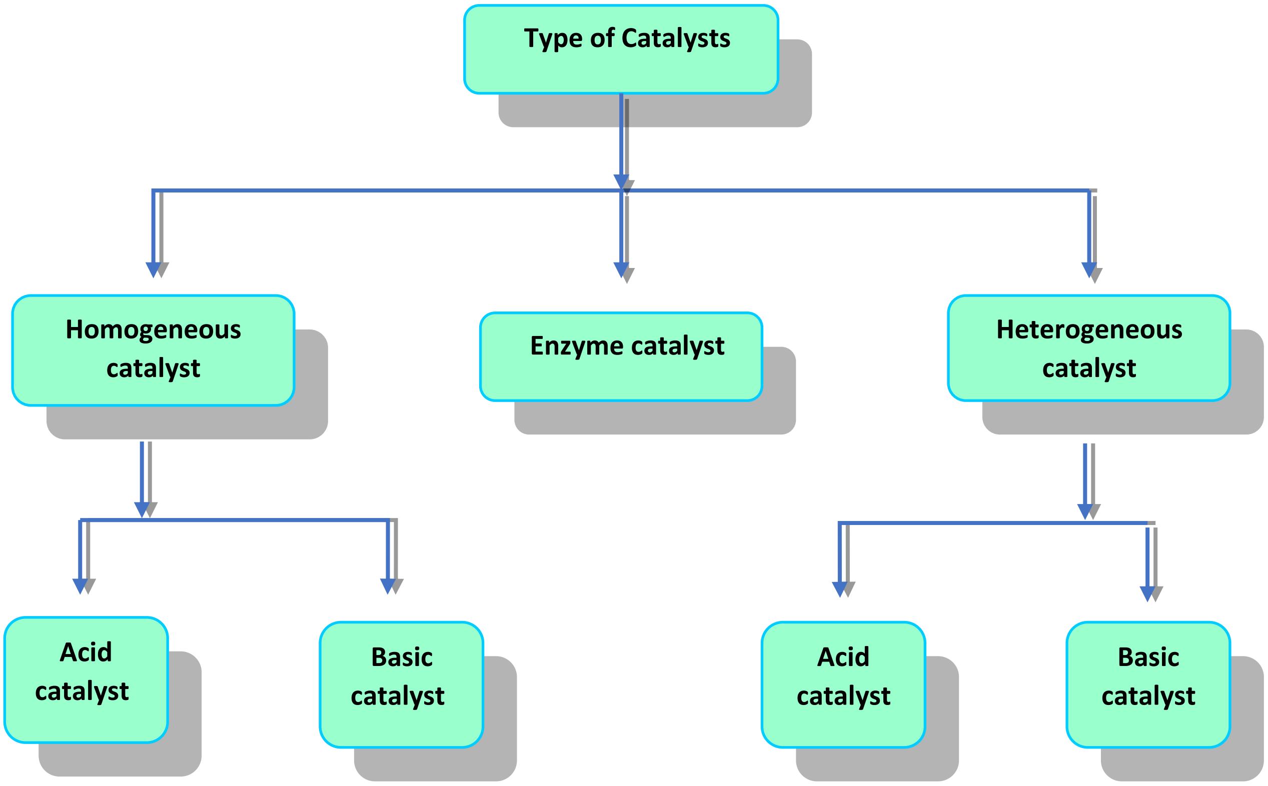


Figure I.12: Classification of the catalyst used in biodiesel production

a. Homogeneous catalyst

The homogeneous catalysts are usually employed to catalyze transesterification reactions for converting triglycerides to biodiesel fuel [34]. As a result, homogeneous:

- **Alkaline homogeneous catalysts:** such as NaOH, KOH, CH_3ONa, and CH_3OK
- **Acidic homogeneous catalysts:** such as $Fe_2(SO_4)_3$, HCl, H_2SO_4

As a result, homogeneous alkaline catalysts are more often a process results in a high-purity, high yield of biodiesel within a short reaction time [35,36]. However, it is limited to the use of oil with less than 0.5 (wt.%) of free fatty acid (FFA), because FFA can easily react with base catalysts and form soap as a byproduct, which will make separation of the product difficult [35]. Sahar et al, [37] were focused on the conversion of WCO into biodiesel. The catalyst does effect was also checked on FAME yield. The KOH concentration was checked in the range of 0.5 to 2% of the oil weight. Fatty acid methyl ester (FAME) yield was 94% in the presence of a 1% catalyst at 50°C.

b. Heterogeneous catalysis

In recent years, heterogeneous catalyst has attracted immense attention for biodiesel production. Heterogeneous catalysts mostly appear in a solid form; thus, the reaction mixture and the catalyst are in a different phase as they can be tailored to match specific requirements and be easily recovered and reused. Heterogeneous or solid catalysts can be grouped into two categories:

- **Basic heterogeneous catalysts:** CaO، MgO،, ZnO.
- **Acidic heterogeneous catalysts:** $H_nXM_{12}O_{40}$ ،Fe_2O_3–SiO_2, Fe–Mn–MoO_3/ZrO_2. [20,38]

c. Enzymatic catalysis

Enzymatic catalysts are biological catalysts, where the enzyme catalyst has attracted great interest in biodiesel production because of its rapid decomposition in the environment because it has a protein structure, and it requires a low temperature to give high productivity however it but it requires a longer time to obtain a good yield (95%). There are several types of enzyme catalysts, but differ according to the type of biomass and the interaction of the user [38,39].

d. The advantages and disadvantages of catalyst

Each different type of catalyst used in the process of transesterification has the advantages and disadvantages as shown in table (I.2)

Table I.2: The advantages and disadvantages of catalyst

catalyst	Advantages	Disadvantages
Homogeneous catalyst		
Basic	High motivational activity • Faster reaction time • Low cost	• Hypersensitive to water and FFA (more than 1%) • Soap formation as a side reaction • Catalyst corrosion is not recyclable
Acid	• Insensitive to the water content in oil and free fatty acids • Avoid soap making	• Long reaction time and slow reaction rate • The catalyst is corroded and not recyclable • High requirements for alcohol and reaction temperature
Heterogeneous catalyst		
Basic	Non-corrosive • Recyclable • Easy to separate	Highly sensitive to water and FFA (more than 1%) • Soap formation as a side reaction • The cost of producing the catalyst is high
Acid	Insensitive to the water content in oil and free fatty acids • Recyclable and environmentally friendly • Non-corrosive	• Long reaction time and slow reaction rate • High requirements for alcohol/oil, reaction temperature, and pressure • The cost of producing the catalyst is high
Enzyme catalyst		
	• Insensitive to the water content in oil and free fatty acids • Avoid soap making • Non-polluting recyclable	The reaction rate is too slow • High cost • Hypersensitive to alcohol

I.3.4.2. Influence of water and free fatty acids

In alkali-catalyzed transesterification, even if water-free vegetable oils and alcohol are used, a certain amount of water is formed by the CH_3ONa solution because of the interaction between NaOH and methanol [40]. The presence of water leads to the hydrolysis of oils to FFA. Figure (I.2) shows water hydrolysis of fats and oils to form free fatty acids. The FFA react with alkaline catalysts to produce soaps formation. Figure (I.13) presents soaps formation in homogeneous alkali-catalyzed transesterification. Soaps formation consumes the catalyst which reduces the biodiesel yield and affects the quality of the product [34,41].

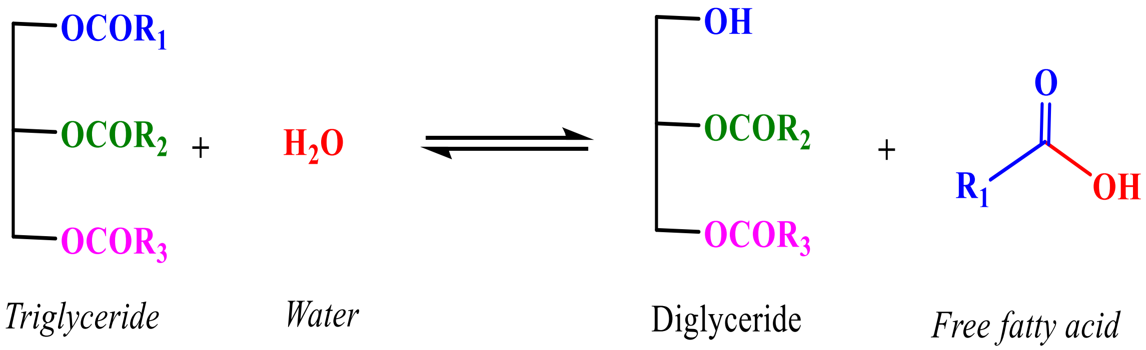


Figure I.13: Hydrolysis of Triglycerides to Form Free Fatty Acids (FFA)

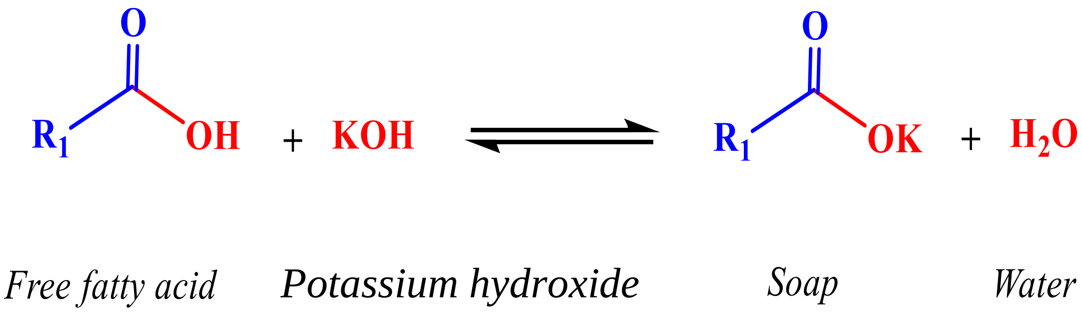


Figure I.14: Soap formation in transesterification by the basic homogenous catalyst.

A two-step reaction process (acid esterification followed by alkali transesterification) was developed to reduce the reaction time and lower the cost by using acid esterification as the first step to get rid of high FFA content [35]. Wang et al. [42], found that ferric sulfate is a good acid catalyst in the esterification of FFA in waste cooking oil. The total two-step reaction conversion of FFA of waste cooking oil into FAME (fatty acid methyl ester) in the two-step method was 97.22 % [42].

I.3.4.3. Effect of rate and type of alcohol

One of the most important parameters affecting ester yield is the molar ratio of alcohol to triglycerides. The transesterification requires the presence of three moles of alcohol and one mole of triglycerides, thus obtaining three moles of fatty acid esters with one mole of glycerol [43]. The ratio of alcohol to oil is always positive, affecting the conversion of biofuels. as reactant concentration is increased, the rate of product formation increases. Therefore, if the alcohol concentration is automatically increased, the rate of substance formation will be accelerated [44]. The maximum ester conversions for vegetable oil and waste cooking oil were found at the methanol to oil molar ratio of 9:1. Table (3) shows the effect of methanol to oil molar ratio on the conversion of oil to biodiesel [45].

I.3.4.4. Effect of reaction temperature

The reaction rate and the efficiency of the transesterification process are strongly affected by the reaction temperature. The viscosity of the oil decreases with increasing temperature which accelerates the reaction rate due to the energy supplied for the reaction [31]. The transesterification process is usually carried out at the boiling temperatures of methanol and ethanol are respectively 65°C and 78°C. Methanol is widely used in this process, having the reactions are generally carried out at 60°C with methanol and 70-75°C with ethanol [31]. Previous studies showed the effect of different reaction temperatures from 60 to 80 °C, the maximum FAME was up to 99.342% at 70 °C [46].

I.3.4.5. Effect of reaction time

Temperature and reaction time are closely related parameters. As we have just seen in the previous paragraph, a temperature rise accelerates the reaction, which also means that the reaction time is shortened [31]. Dena A. et al. [47] in this research, biodiesel generated from waste cooking oil is discussed to explore the effect of time using conventional (CV) process, different reaction times ranging from 30 to 180 minutes for biodiesel yield were studied under the percent biodiesel conversion reached a peak value after 120 minutes. Silitonga A. et al. [48] compared to traditional heating systems, the yield was found to be 95.42% at reaction time in microwave reactors that transform triglycerides in oils from *Ceiba pentndra* into biodiesels in a quicker time:388 s.

Table I.3: The previous study for biodiesel production

No.	Oil type	Cat.	M:O	Cat.	T(°C)	t(min)	Y (%)
1	Cotton seeds oil [49]	KOH	6: 1	0.6	55	60	96
2	Palm oil [50]	KOH	8: 1	2	55	300	98
3	Soy oil [20]	NaOH	6: 1	1	60	60	96
4	Palm oil [51]	NaOH	6: 1	1	65	120	96
5	Waste cooking oil [52]	KOH	9: 1	1	60	50	93
6	Waste frying oil [46]	NaOH	6.5: 1	1.013	70.1	60	99.34
9	Waste cooking oil [53]	KOH	8:1	1	/	6	96.8
10	Waste cooking oil [54]	NaOH	6:1	1	60	5	98.87
11	Waste cooking oil [55]	NaOH	12:1	0.8	65	2	98.2
12	Waste cooking oil [56]	NaOH	5:1	2	65	30	94.6
13	Waste cooking oil [57]	KOH	12:1	1	40	30	90
14	Waste cooking oil [14]	H_2SO_4+ KOH	6: 1	1	60	60	95.28
			3: 1	0.5	75	45	
15	Waste cooking oil [37]	H_2SO_4+ KOH	2.5: 1	0.2	50	6h	90
			3: 1	1	60	60	
16	Sunflower oil [20]	CaO	3: 1	3	60	120	94
17	Palm oil [58]	TiO2	20: 1	3	45	45	90.21
18	Waste cooking oil	CaO	15:1	4	50	60	95
19	Soy oil [20]	SrO	6: 1	3	70	30	95
20	Palm oil [59]	CaO	12:1	4.5	70	120	98.2
21	Waste cooking oil	CaO/AC	25:1	/	60	480	94
22	Palm oil [20]	CaO/AC	40: 1	11	120	420	90

M:O: Molar ratio Methanol:oil, Cat: Catalyst, T: Temperature, t: Reaction time, Y: Biodiesel yield,

I.4. Bioethanol

Bioethanol is a biofuel that is produced from biomass by fermenting sugary materials (such as sugar, starch, agricultural and urban waste). It is considered one of the most important ways to reduce both the consumption of crude oil and environmental pollution because it gives way to mixed fuels in an engine [60]. The bioethanol produced can be used directly as a substitute for gasoline in engines, as it has different properties from gasoline. and table (I.4) shows the physicochemical properties of bioethanol [61].

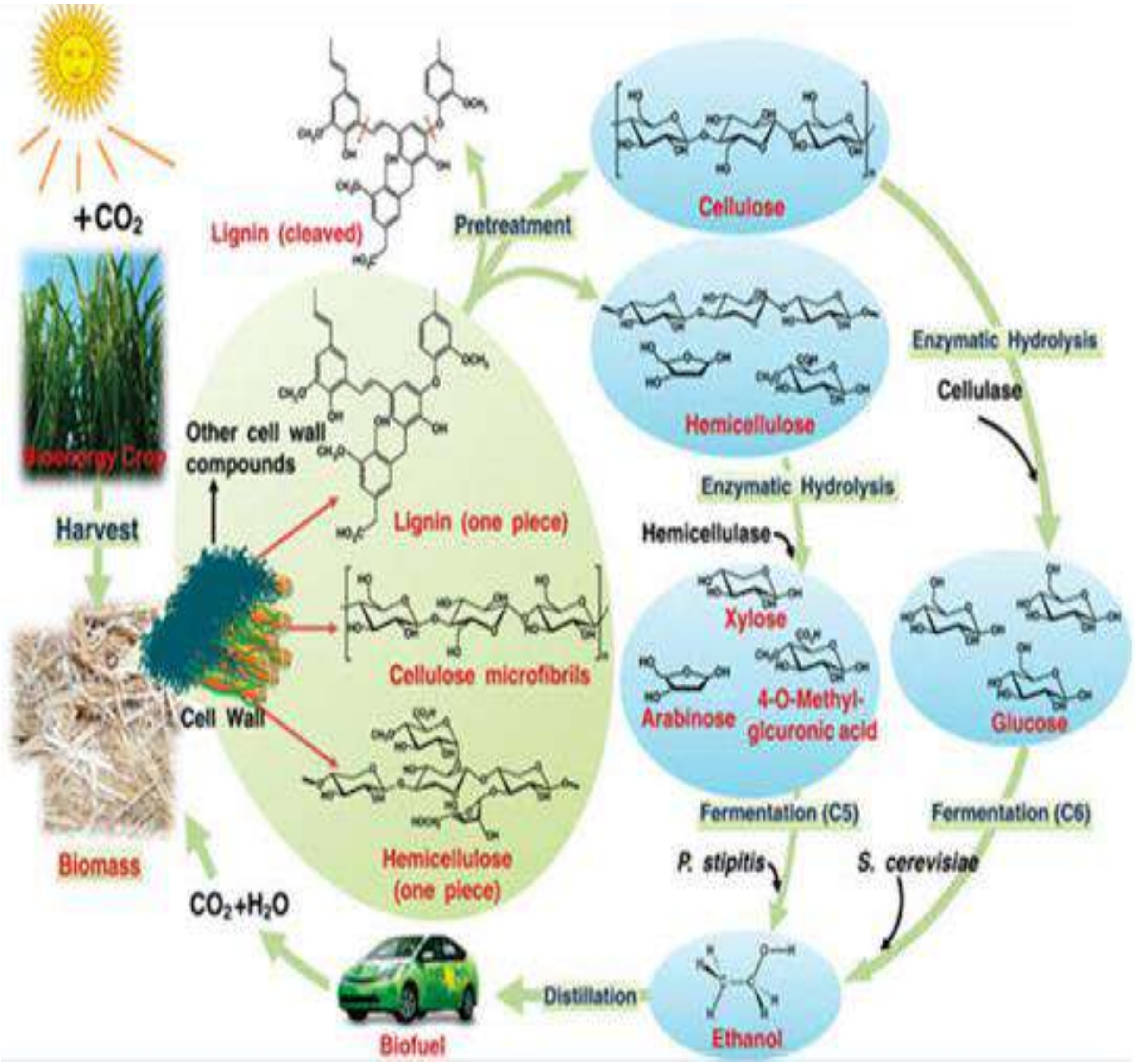


Figure I.15: Bioethanol fuel production from renewable biomass

Table I.4: The physicochemical properties of bioethanol

Properties	Bioethanol	Petrol (Gasoline)
Chemical formula	C_2H_5OH	C_nH_{2n+2} (n=4-12)
Molecular mass (g/mol)	46.07	100-105
Physical condition	Transparent liquid	Light yellow liquid
Octane number	108	88-100
Boiling point (°C)	78	27-225
Flash point (°C)	13	-43
Freezing point (°C)	-96.1	-22.2
Auto-ignition temperature (°C)	440	275
Density ((Kg/m3, at 298K)	0.79	0.69-0.79

I.4.1. Biomass of bioethanol

There are several main sources of bioethanol production, they can be classified as follows [62]:

- ✓ **Sugar materials** (sugar cane, sugar beet, corn, fruits, industrial waste)
- ✓ **Carbohydrates:** - Cereals (corn, wheat...), root crops (potatoes...)
- ✓ **Cellulosic materials:** wood, agricultural and industrial waste (straw, firewood, paper...)

I.4.2. Bioethanol production method

I.4.2.1. Pretreatment

Pretreatment has a significant impact on the amount of ethanol production and production cost, which makes hydrolysis easier and produces more fermentable sugars. There are several methods currently used for pretreatment, as shown in the following figure I.16 [63,64]

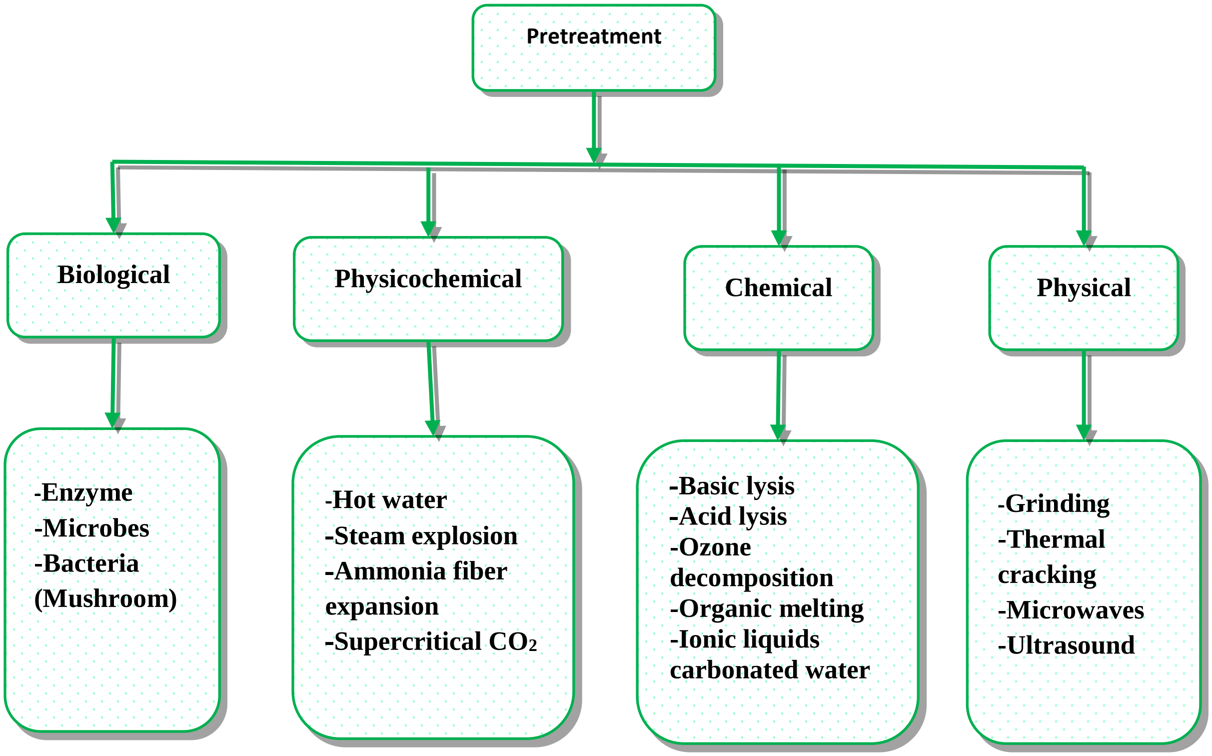


Figure I.16: Types of methods used for biomass pretreatment.

a. Physical Pretreatment

Physical pretreatment of lignocellulose normally includes the comminution (milling, grinding, chopping, etc.) method which leads to particle size reduction, depolymerization, and lowering of its crystallinity. This structural alteration results in improved cellulose reactivity for subsequent biochemical conversion [65,66]. Mechanical approaches are not usually suitable when applied individually [67]. Mechanical pretreatment that is associated with grinding has the advantage of being eco-friendly, as it does not rely on using chemicals such as alkalis or acids. Nevertheless, it is also an energy-intensive operation, therefore, better to be used in combination with other pretreatment techniques to save energy and lower the costs of the process. Mechanical treatments are not capable of removing lignin, which acts as both a physical and chemical barrier to limit enzyme access to cellulose [68,69]. Different (dry/wet, centrifugal, jet, and vibratory) ball milling and compression milling have been used in a variety of research work alone [70]. He compared the effects of four mechanical deconstruction methods namely ball mill, vibratory ball mill, centrifugal mill, and jet mill in terms of energy demand and energy efficiency at lab scale on the enzymatic hydrolysis of lignocellulosic biomass for bioenergy production. They reported the highest glucose yield were achieved when using the vibratory ball mill for 3 h and in term of energy efficiency, the centrifugal mill method was the most efficient one compared to the ball mill, vibratory ball mill, and jet mill [71].

b. Chemical Pretreatment

Chemical pretreatment commonly done by using a solvent or a combination of reagents, simplifies the conversion of lignocellulose materials by reacting with the biomass to either has a physical (via solvation, disrupting covalent and hydrogen bonding) or a chemical (via chemical reactions) effect on the structure of biomass [72,73]. Acidic [71], basic (alkaline), carbon dioxide treatment [73], ionic liquids, organosolv [74], and ozonolysis [75]are all diverse chemical pretreatment techniques, that impact the biomass properties. Compared with the other pretreatment methods (physical and biological), chemical pretreatment is the most predominantly used method because they are usually less expensive and result in faster rates and better efficiencies in enhancing the degradation of complex organic materials [76,77] Nevertheless, chemical pretreatment methods might have their own limitations or weaknesses, including corrosivity of acids and bases especially the strong types such as concentrated acids, handling, and the need for recovery steps after pretreatment to make the pretreatment procedures economically viable [76,78].

c. Physicochemical Pretreatment

Physicochemical pretreatment of lignocellulosic biomass is a combination of both physical and chemical treatment steps as an effective pretreatment method aiming to convert lignocellulosic materials into monosaccharides by enhancing the removal of lignin components and increasing the efficiency of the hydrolysis process [79].

Although this technique shows effective enhancement of the enzymatic hydrolysis of lignocellulose; however, they may produce degradation byproducts such as furan derivatives, weak acids, and phenolic and inorganic substances that can inhibit the hydrolysis of sugar and fermentation process [80,81]. In addition, these kinds of pretreatments depend on the conditions and solvents used, which influence the biomass' physical and chemical properties [82].

d. Biological Pretreatment

Unlike physical, chemical, or physicochemical pretreatment procedures, the biological pretreatment of lignocellulosic biomass is assumed to be the eco-friendliest technique with mild environmental conditions and low capital which does not require 72 conditions (air as gas for 90 s with 60% dry matter) resulting in high conversation yield of glucan (90%), xylan (69%), and very high yield of biomass/sugars recoveries (84%) after the subsequent enzymatic hydrolysis high energy, therefore, has low operational cost [69,80]. Furthermore, they have also indicated that for obtaining the maximum yields of glucose, the duration of plasma pretreatment should not be more than 90 s [85].

e. The advantages and disadvantages of pre-treatment methods

To conclude, there are four pre-treatment methods, and each method depends on several techniques, and each technique has its advantages and disadvantages, as shown in the table I.5 [72,73].

Table.I.5: Advantages and Disadvantages of pre-treatment methods

Pretreatment p.		Sugar y.	E. cost	Advantages	Disadvantages
Physical	Comminution	Low	High	Friendly (not depend on using chemicals), increases surface area and reduces cellulose crystallinity	Energy-intensive procedure, not capable of altering or solubilizing lignin structure
Chemical	Acid pretreatment	High	High	Increase of accessible surface area, solubilization of hemicellulose; alters lignin structure	Corrosive, hazardous, and toxic, need equipment that is resistant to corrosion, needs neutralization step to remove acids from substances
	Alkaline pretreatment	High	/	Increase of accessible surface area and porosity, selectively for breaking down the lignin structure without degrading carbohydrates	A considerable portion of lignin may still be combined with cellulose even after pretreatment and may suppress cellulase enzymes in subsequent enzyme hydrolysis. needs neutralising step to eliminating lignin and the inhibitors
	Supercritical Carbon	/	/	Increase of accessible surface area, green pretreatment procedure because CO_2 is a non-toxic solvent, Increase in digestibility of biomass Increase of accessible surface area, reduces cellulose crystallinity, non-flammability	Costs and expenses of instruments which can be able to endure high-pressure conditions of pretreatment, not capable of altering or solubilizing lignin structure
	Ionic Liquids (ILs)	/	/	Properties, good chemical, and thermal stability, most of the ILs are recoverable and reusable. Increase of accessible surface area, hydrolyses hemicelluloses, convenient	Pricey and toxic to microorganisms and hydrolytic enzymes Pricey, unrecovered solvents may cause the production of inhibitory compounds, due to the
	Organosolv	High	High	Restoring of solvents using simple distillation, recycling back the solvents to the process, significant delignification	higher volatility and flammability of the organic solvents pretreatment process needs to be conducted under highlyrestricted environments
	Ozonolysis			Increase of accessible surface area, solubilization of hemicellulose, altering or solubilizing lignin structure production of no	High operational costs due to requirements of large amounts of ozone

Physico-chemical	Steam Explosion	High	High	Increase of accessible surface area and porosity, solubilization of hemicellulose, altering or solubilizing lignin structure Increase of accessible surface area, Hydrating cellulose, solubilization of hemicellulose,	Formation of some inhibiting by-products (e.g. aromatic compounds, furfural and HMF) The process is carried out under high
	Hydrothermol -ysis	High or Low	High	somewhat degrading lignin, environmentally friendly process, handle biomass feedstock with high moisture content (avoids the need for a dewatering step before treatment)	temperature and high pressure, resulting in an increase in energy consumption and process cost
	WetOxidation (WO)	High or Low	High	Increase of accessible surface area, avoids the need for a dewatering step before treatment, solubilization of hemicelluloses and lignin, low temperatures process	Consumption of large amounts of energy due to using pure oxygen, the unselective characteristic of all WO procedure bears the risk of substantial losses in the level of organic matter (e.g., hemicellulose)
	Ammonia Fibre/Freeze	High	High	Increase of accessible surface area, reduces cellulose crystallinity, capable of altering and solubilizing lignin structure, it allows much	Not ideal for high lignin content biomass, moreover, compared to dilute acid pretreatment it is not solubilized hemicellulose
	Explosion (AFEX)	High	High	Higher loadings of solids in the fermentation procedure due to being a dry-based method	to any great extent
	Plasma Pretreatment	High	High	Increase of accessible surface area, absence of toxic and polluting substances, considered as low-temperature pretreatment process, reduces cellulose crystallinity, destruction of the hemicelluloses complex, significant	Plasma pretreatment's energy conservation and economic sustainability have yet to be fully piloted. , low reaction control
Biological	Fungi pretreatment	Low		Increase of accessible surface area, solubilizing lignin structure, the eco-friendliest technique with mild environmental conditions does not require high energy, therefore, low operational cost	The pretreatment results in carbohydrate loss, therefore, it is required to utilize the relevant type of fungal strain/biomass combination, low rate of hydrolysis

I.4.2.2. Fermentation

a. Alcoholic fermentation

Fermentation is a biological process that converts sugars (such as glucose, fructose, and sucrose) into ethanol and carbon dioxide and produces energy by microorganisms (yeast or bacteria), and it is an anaerobic process because yeasts perform this conversion in the absence of oxygen [88]. Many yeasts perform the fermentation process, but the most widely used is bread yeast (S*accharomyces cerevisiae*) [89].

b. The yeast *Saccharomyces cerevisiae*

The yeast *Saccharomyces cerevisiae* occupies a privileged place in industrial activities. It has been used by humans for millennia for the production of beverages and fermented products (wine, beer, bread). It plays a very important role in the food industry as a fermentation agent and for the development of derived products. Nowadays, yeast is also widely used as a cell factory for the production of molecules of interest. In the pharmaceutical and medical fields, it is used for the production of vaccines, probiotics, or proteins such as insulin. It also plays a key role in the chemical industry for the synthesis of commodities such as lactic acid for the production of plastics and in the field of renewable energies and biofuels (bioethanol) [88,89].

c. Alcoholic fermentation reaction

Glucose is naturally converted directly into ethanol by a variety of microorganisms through a series of chemical reactions as shown in figure I.17.

1. **The first step:** The breakdown of sugar (glucose) into pyruvate:
    1. Converting 6-carbon glucose into two 3-carbon molecules of pyruvate using an enzyme (aldolase) and producing energy (ATP) for feeding by yeast.
2. **The second step:** Converting pyruvate to ethanol. This is a two-stage process:
    2. The removal of carbon dioxide (CO_2) from pyruvate, which leads to the formation of acetaldehyde, and the enzyme responsible for this reaction is called pyruvate decarboxylase.
    3. The acetaldehyde is then converted to ethanol by another enzyme (alcohol dehydrogenase (ADH)) [90].

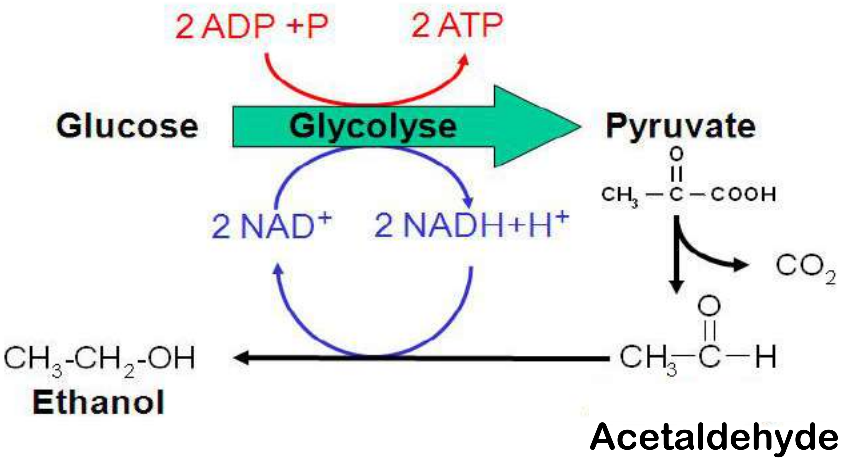


Figure I.17: The reaction mechanism of the fermentation of glucose into bioethanol

I.4.2.3. Effect of condition on the alcoholic fermentation:

a. Effect of yeast quantity

The concentration of yeast in the fermentation reaction is affected by the concentration of sugars. The higher the concentration of sugars, it leads to a stabilization of the fermentation rate because it exceeds the ability to absorb the cells of microorganisms. The concentration of yeast has a direct relationship to the concentration of sugars to give high productivity of bioethanol, but it is not the only factor that controls the productivity of its temperature. A role also because these organisms do not live-in high temperatures [88,89,91].

b. Effect of reaction temperature

The rate of growth of microorganisms is directly a ff elected by temperature. A high temperature, unfavorable to cell growth, becomes a stress factor for microorganisms [88]. The ideal temperature range for fermentation is between 20 and 35°C. Free cells of S*accharomyces cerevisiae* have an optimum temperature close to 30°C while immobilized cells have a slightly higher optimum temperature due to their ability to transfer heat from the surface of the particles to the interior of the cells [91].

c. Effect of pH

Ethanol production is affected by the pH of the reaction medium because it affects yeast growth, fermentation rate, and product formation. The permeability of some essential nutrients to cells is affected by the H^+ concentration in the fermentation reaction, in addition, the survival and growth of yeasts are affected by the pH [92]. In fermentation with *Saccharomyces cerevisiae*, the optimum pH for ethanol production is 4.0 to 5.0. It was observed that a pH of less than 4.0 leads to a longer incubation period and a lower ethanol concentration. However, if it is above 5.0, the ethanol concentration drops dramatically [91].

d. Effect of fermentation time

Fermentation time affects the growth of microorganisms. A shorter time leads to inefficient fermentation due to reduced growth of microorganisms [92]. On the other hand, a very long fermentation time results in a toxic effect on microorganism growth due to the high concentration of ethanol produced by fermentation. complete fermentation can be achieved at a lower temperature using a longer fermentation time which results in lower ethanol yield [88,93].

Table (I.6) shows the effect of fermentation condition on the conversion of different biomass to bioethanol.

Table I.6: The previous study for bioethanol production

Feedstock	**Yeast strain**	**Fermentation condition**	**Yield (g/l)**	**Ref.**
Sugar molasses	*S. cerevisiae*	30°C, 115rpm, 12h	78.6	[94]
Cane molasses	*S. cerevisiae* M30	33°C, 150rpm, 48h	92.0	[95]
Glucose and sucrose	*Mutantbaker*'syeast3013	30°C, 16h, 28h	92.7	[96]
Sugarcane bagasse	*S. cerevisiae* MTCC174	30°C, 72h,96h	15.4	[97]
Sugar beet thick juice	*S. cerevisiae* DTN	30°C, 48h	52.3	[98]
Sorghum juice	*S. cerevisiae* NP01	30°C, 72h, 96h	98.5	[99]
Cornmeal	*S. cerevisiaevar. ellipsoideus*	30°C,74h, 150rpm	88.9	[100]
Wheat straw	*S. cerevisiae* T0936	30°C, 96h, 150rpm	37.1	[101]
Cassava starch	*S. cerevisiae* CHY1011	32 °C 4.5, 66h,120 rpm	89.1	[102]
Corn stover	*S. cerevisiae* K35	30°C, 5.5, 72h, 120 rpm	41.2	[103]

Wood	*S. cerevisiae*	30°C, 5.5, 16h,150 rpm	18.52	[104]
Reed	*A. cerevisiae* ATCC	38°C, 5.0, 96h,150 rpm	55.0	[105]
Sweet potato	*S. cerevisiae*	30°C, 5.3, 24h, 150 rpm	128.5	[106]
Date (Ghars)	*S. cerevisiae*	32°C, 4, 72h	346.66	[89]

I.4.2.3. Purification of bioethanol

After the fermentation process, followed by the process of purifying bioethanol on the water by distillation. It is a chemical technique used to separate the different components in the mixture. Ethanol can be separated from water by distillation because the boiling point of ethanol is lower than water (79 °C), but the purity of this method is 92% to 95% [107].

I.5. Conclusion

In conclusion, biofuels have become attracting increasing interest around the world to reduce greenhouse gas emissions and environmental pollution compared to fossil fuels responsible for 73% of global carbon dioxide emissions, which is the main factor contributing to global warming and environmental change. The best way to produce biodiesel is transesterification because it requires the least cost. As for bioethanol, it is produced by fermentation. From this, we will discuss in the coming chapters the valorization of some low-cost waste materials and materials for biodiesel production by esterification method and bioethanol by fermentation.

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Chapter II

Biodiesel preparation methods

II.1. Introduction

The biodiesel is an alkyl ester combination of long-chain fatty acids that can be produced with various methods from a wide range of feedstocks obtained from animal fats or vegetable oils by esterification reaction with alcohol in the presence of a catalyst [1,2]. Among others, as to the economic aspect, biodiesel is produced using low-cost oils which is the case of this study [3]. Transesterification process is the most popular and cost-effective method of obtaining biodiesel [4].

In this study, waste cooking oil (WCO) was used to produce biodiesel because it depleted it in nature and so returned these worthless materials to the consumption cycle via green fuel production. In this chapter, biodiesel was produced by three methods with two catalysts, and in the end, we compare the quality of biodiesel with international standards.

II.2. Experimental plan

The general diagram adopted for the realization of this study is summarized by the figure (II.1) below:

II.2.1. Sample preparation

II.2.1.1. Sample collection

Waste cooking oil (WCO) was selected for this study because it leads to environmental pollution when exhausted in nature. The sample was collected from sweets shops in the city of Ouargla.

II.2.1.2. Sample preparation

1. Filter and wash with water the oil from impurities using a piece of cloth.
2. heating the oil for 70°C for two hours to remove traces of water.
3. After we cool down, we add Na_2SO_4, filter paper was filtered

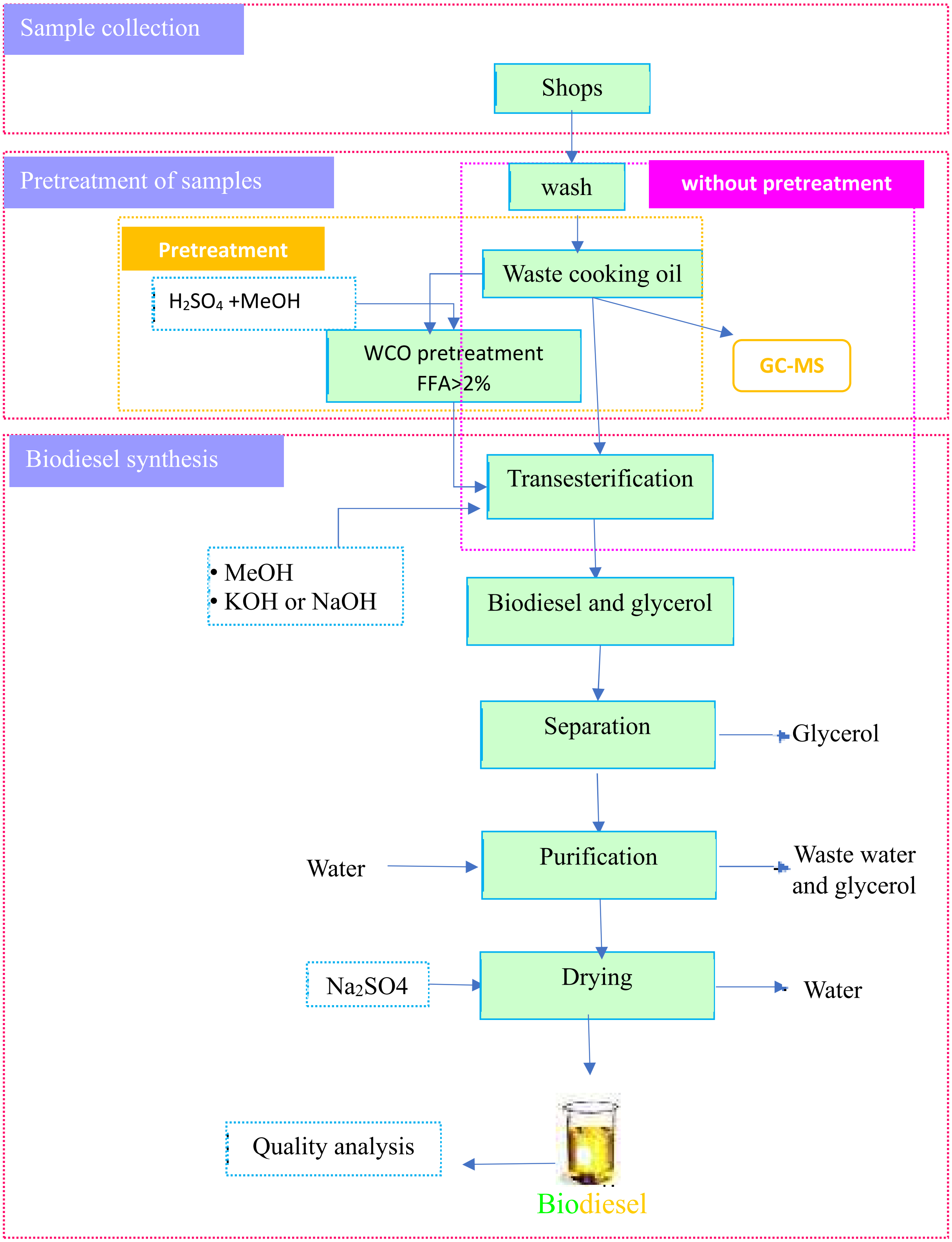

Figure II.1. Scheme of biodiesel synthesis

II.3. Transesterification

Transesterification is the traditional technique most used for the production of biodiesel. During this reaction, vegetable oils react with methanol molecules to form methyl monoesters and glycerol [5]. In this present study, we applied this method to waste cooking oil and date seed oil to obtain biodiesel. Three methods of oil esterification were used:

- ✓ Conventional method (CV)
- ✓ Microwave-assisted method (MW)
- ✓ Ultrasound-assisted method (UT)

The effect of changing the reaction conditions on the esterification reaction and improving productivity will also be studied. Two methods: the normal (conventional) method and the microwave method, we used:

The conditions under which the study will be conducted are as follows:

- ✓ Effect of molar ratio effect (methanol/oil) on interaction.
- ✓ Effect of type and concentration catalyst
- ✓ The effect of temperature on the reaction.
- ✓ The effect of reaction time on the reaction.

II.3.1. Biodiesel synthesis protocol

The biodiesel synthesis protocol consists of the following steps (figure II.2) [6,7].

II.3.1.1. One-step transesterification

Biodiesel preparation method by one-step transesterification:

- ❖ **The reaction:**
  1. We used the MeOH-oil ratio molar 3:1 and (1% wt.) as catalysts (NaOH, KOH)
  2. The oil dissolve of methanol mixture in the reactor at 60°C from 6 min, 800 rpm.
  3. Introduce a thermometer from the neck of the flask, leave the reaction for two hours.
- ❖ **Separation phase:**
  4. Allow glycerin and biodiesel to separate into two phases for one day in a separatory funnel.
  5. Next, pour the glycerin into a bottle and leave the esters in the separatory funnel.

- **Washing:**

6. Add 10 ml of the water to get rid of the remaining alcohol and catalyst (wash).
7. Recover the biodiesel and add 1 to 2% of its mass of sodium sulfate to absorb the remaining moisture.
8. Finally, filter and recover the biodiesel.

II.3.1.2. The two-step's transesterification

Biodiesel preparation method by two-step transesterification:

- **The first step:**

1. We used the MeOH-oil ratio molar 9:1 and sulfuric acid (1% v/v) as catalysts.
2. The oil dissolve of methanol mixture in the reactor at 60°C from 6 min to 800 rpm.

- **The second step:**

3. We used the MeOH-oil ratio molar 6:1 by molar, 1% of catalyst (NaOH, KOH).
4. The oil-Dissolve of methanol- mixture in the reactor at 70°C from 6 min by 800 rpm.
5. After that, the residual water and methanol from the biodiesel are removed using the same method as before [7-9].

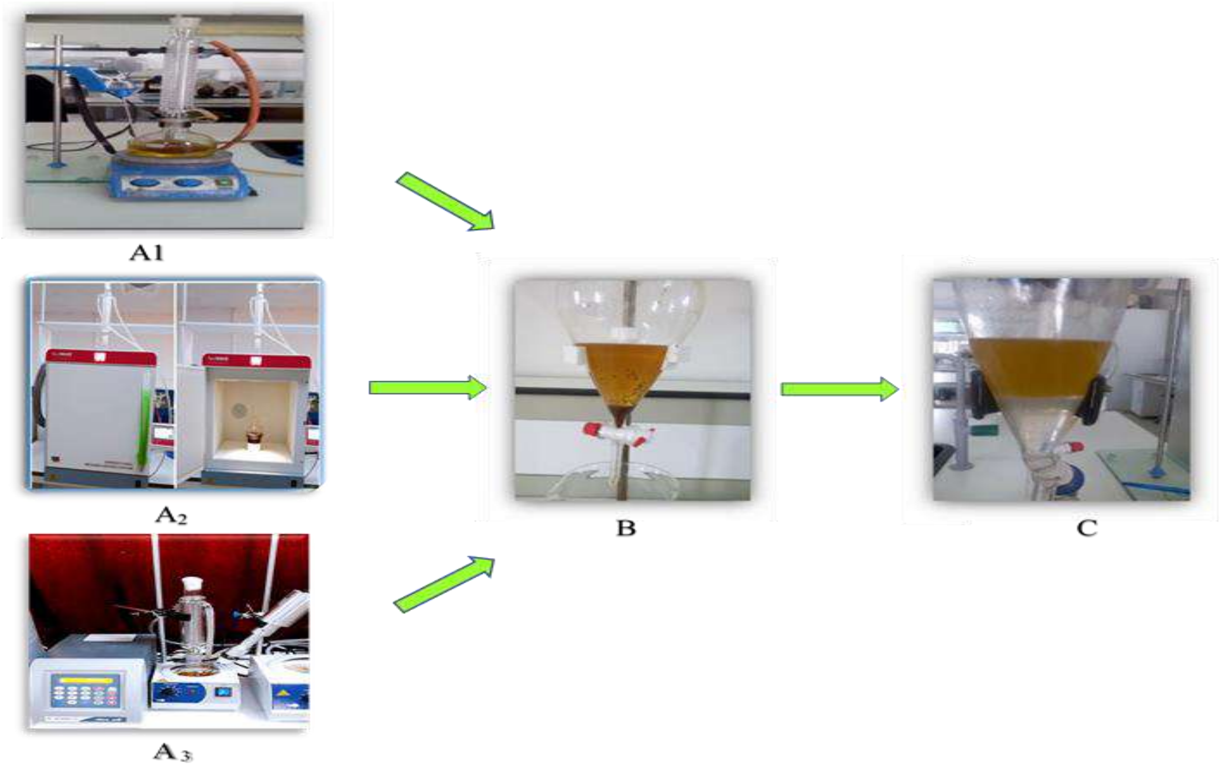

Figure II.2: Biodiesel preparation method: (A) Synthesis, (B) Separation and (C) Purification

II.3.2. Analysis of bio-oil and biodiesel

II.3.2.1. GC-MS analysis of fatty acid

Gas chromatography coupled with a mass spectrometer (GC-MS) is the most widely used analytical method to determine the many fats and volatile oil compounds.[5] In addition, the potential of GC coupled to MS. This technique is reserved for the analysis of easily vaporizable and thermally stable molecules is well known for the determination of volatile compounds contained in complex samples of perfumes, allowing the identification of complex sample compositions.[5,10]

- **GC/MS operating conditions**:

Gas chromatographic analysis was performed on a Shimadzu TQ8040 NX apparatus. The operating conditions are collated in Table (II.1) [11].

Table II.1: Operating conditions for GC/MS analysis.

GC Program	
Capillary column	30 m, ID: 0.25 mm, film :0.25 µm
Injection Temperature (°C)	250 °C
Ion Source Temperature (°C)	200 °C
Carrier gas (rate of flow)	1.21 mL min^{-1} (Helium)
Oven Program	60 to 300°C ,10°C/min
Injected quantity	0.5µL
Injection Mode	Split
MS Program	
Mass spectra	45 –500 m/z
	30–35 min

Figure II.3: GC-MS TQ8040 NX apparatus (Shimadzu)

II.3.2.2. Fourier transform infrared spectrometer (FTIR)

Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. the apparatus of FT-IR used in our work is (FT-IR Cary660 (Agilent tech)), It shows in figure (II.4) [12,13].

Figure II.4: (FT-IR Cary660 (Agilant tech)) apparatus

II.3.2.3 Density

Density is the ratio of the density of a liquid to that of water under a fixed pressure and temperature waves. The measurement temperature for oil and fuels is 15°C according to international standards. The density of fuel has an important effect on engine performance, such as certain engine properties such as cetane number, viscosity, and calorific value which are strongly related to density. A fuel having a high density leads to a greater mass of injected fuel, as a consequence of an increase in fuel consumption. Ester density depends on molar mass, free fatty acid content, water content, and temperature [14,15]. The density value is determined using an apparatus of viscometer (Anton Paar 3001.) (figure II.5).

II.3.2.4 Viscosity

Viscosity is an important characteristic of fuels, it directly influences combustion and affects engine performance and pollutant emissions, and the ease of starting the engine. High viscosity leads to poor spraying which is related to poor volatility causing incomplete combustion in the engine. This poor combustion results in degraded engine performance and higher pollutant emissions. The viscometer used in our work is a vibration viscometer (figure II.5) [5,16,17].

Figure II.5: Viscometer (Anton paar 3001) apparatus

II.3.2.5. Determination of acid number (AN)

The acid number is the number of milligrams of soda (NaOH) needed to neutralize the free acids in 1 g of fat. The acid number is measured by the assay or titration method. This involves reacting the solution containing the reagent to be titrated with a solution containing the titrating reagent, the concentration of the reagent is known. The acid value was determined with ASTM D 1298 [17,18]. The acid value of the oil was deduced with Equation (1) as follows:

$$\boxed{AV = 56.1 \times N \times V/W \quad \dots\dots\dots (1)}$$

Where:

W: Weight of the oil sample in g of the test portion

V: Volume expressed in ml of 0.1 N solution of ethanolic KOH.

N: Concentration of sodium hydroxide NaOH

- **Operating mode:**
  1. Weigh with a balance 1 g of the oils in a 250ml erlenmeyer flask, and record the mass precisely.
  2. Take 10 ml of methanol using a pipette and add to the erlenmeyer flask and mix well.
  3. Add a few drops of phenolphthalein to the solution as a colored indicator.

4. Titrate with an ethanolic solution of KOH (0.1M) until the color is pink.
5. Accurately record the volume of KOH.

II.3.2.6. Determination of saponification number (SN)

The saponification number is the amount of potassium hydroxide (KOH), expressed in mg required to saponify 1 g of fatty substance. The saponification index (IS) tells us about the behavior of the oil. It reveals that the oil is more or less easily saponifiable, that is to say if this oil will form more esters or else more soaps. This index also takes into account the average length of the fatty acid chains constituting the fatty substance. The saponification number was determined using the indicator method according to ISO 3657 [5,18].

The determination of the saponification was calculated with Equation. (2):

$$SN=56.1\times N\times(V_0-V_1)/W \ldots\ldots(2)$$

Where:

W: Weight of oil (g),

N: Normality of HCl solution,

V_1: Volume of HCl solution used (ml)

V_0: Volume of HCl solution used for the blank (ml)

- **Operating mode:**
  1. Weigh with a balance 0.4 g of the oils in a 250 ml flask, and record the weighed mass accurately.
  2. Add 20 ml of the ethanolic KOH solution (0.2 M) to a flask.
  3. Heat the contents of the flask under reflux in a water bath for 30 minutes from the boil.
  4. After cooling, back-titrated with an aqueous hydrochloric acid solution (1M HCl) in the presence of phenolphthalein, at equivalence, the solution turns pink.
  5. Accurately record the volume of HCl used during the titration.
  6. Under the same operating conditions, the blank is titrated and assayed until the phenolphthalein turns colorless.

II.3.2.7. Determination of iodine number (IN)

The iodine number is a number that determines the number of double or triple bonds in oil or fuel (number of unsaturation). The presence of these types of bonds indicates the ability of this liquid to oxidize or form gums or polymers during long storage times. The iodine value was determined as specified by ISO 3961 [16,17].

The iodine is calculated with Equation. (3) as follows:

$$\boxed{IV=12.69 \times N \times (V_2-V_1)/W \text{ ....(3)}}$$

Where:

N: Normality of sodium thiosulphate

V_1: Volume of sodium thiosulphate (V_1)

V_2: Volume of sodium thiosulphate used for blank (V_2)

W: Mass of the sample

Operating mode:

1. About 0.4 g of the oil was added into a conical flask containing 20 ml of CCl_4 to dissolve the oil.
2. Add 25 ml of ICl reagent to the mixture with the flask covered with a stopper.
3. The flask was kept in the dark for 2.5 h before adding 20 ml of 10% aqueous KI and 125 ml of water.
4. The mixtures were titrated with 0.1 N sodium-thiosulphate solutions until the yellow color disappeared.
5. adding a few drops of 1 % starch indicator and drops of sodium thiosulphate, we have continued the titration with shaking until the blue color vanished.
6. The same method was used for the blank test.

II.3.2.8. Determination of cetane number (CN)

The cetane number of biodiesels was deduced using an empirical equation presented with the result of the saponification number and iodine value of the biodiesel as follows [18]:

$$CN = 46.3 + (5458 / SN) - 0.225\ IV \quad .... \ (4)$$

Where:

CN: Cetane number

SN: Saponification number

IV: Iodine number

II.3.2.9. Determination of flash point

The flash point (FP) is the lowest temperature at which volatiles are produced at a rate that allows them to be ignited when in contact with a heat source: flame or spark. If the heat source is removed, the ignition will stop, but not to support combustion. In other words, it is an indication of the presence of volatile and flammable materials in the fuel. A high flash point indicates that the material is less likely to ignite during storage or handling or transport. The flash point is determined using the Normalab Pensky Martens apparatus [5,17].

Operating mode:

1. About 10 ml of the biodiesel produced was heated at a slow constant rate on a hot plate.
2. After which, a thermometer was inserted into it, and an ignition source was moved over the top of the cup containing the biodiesel sample.
3. The minimum temperature at which the vapor above the liquid ignited was read off as the flash point [18].

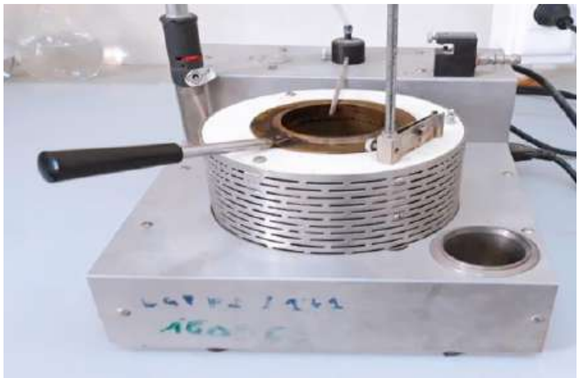

Figure II.6: Normalab Pensky Martens apparatus

II.3.2.10. Determination of cloud point

The cloud point (CP) of a fuel is the lowest feasible temperature at which the wax begins to crystallize and form a cloudy appearance. It is the most typical criterion for setting low-temperature fuel controls. The CP of biodiesels varies greatly depending on the fatty acid composition of the feedstocks used. The cloud point of biodiesel is measured using ASTM D2500 procedures and methyl ester has the lowest CP value of 4°C and the highest value of 19.12 °C [19,20].

Operating mode:

1. About 10 ml each of the biodiesel blends were poured into a 50 ml beaker.
2. They were placed in a refrigerator and were periodically examined.
3. The temperature at which a haze was first observed in the biodiesel blends was noted.
4. These temperatures were recorded as the cloud point [18].

II.3.2.11. Determination of pour point

The pour point (PP) of liquid fuel is the lowest temperature at which the fuel loses its flow characteristics. PP is also an important parameter in cold flow operation because the fuel can only be used above the pour point value. In general, biodiesel has higher CP and PP than conventional diesel. ASTM D 97 specifies the procedure for estimating the PP of biodiesel fuel, with the lowest PP of 18°C and the highest value of 15.5°C [19,20].

Operating mode:

1. About 10 ml each of the biodiesel blends were poured into a 50 ml beaker.
2. They were placed in a refrigerator for them to congeal.
3. When they congealed, they were removed from the refrigerator.
4. They started to melt their various temperatures were read off as the pour point [18].

II.4. Results and Discussion

II.4.1. Analysis of the oil composition

The results of the analysis of the components of the oil using GC-MS are shown in table (II.2), from which it is noted that the percentage of fatty acids in the used oil amounted to 89.91%, with 33.25% for saturated fatty acids and 56.62% for unsaturated fatty acids. From these results, we can deduce the molar mass of this oil, which amounted to (810.35 g/mol) using Equation (4) as follows [17]:

$$\boldsymbol{M_{oil} = (M_{acid} \times \alpha_i) + M_{gly} - M_{H2O}} \quad \ldots. \ (4)$$

Where:

M_{oil}: Molar mass of oil

M_{acid}: Molar mass of fatty acid

∝i: Percentage of fatty acid in the oil

M_{H2O}: Molar mass of water (18g / mole)

M_{gly}: Molar mass of the glycerol molecule (136 g / mole)

Table II.2. Composition of fatty acids in waste cooking oil by GC-MS

Fatty acid	Molecular formula	Molar mass (g/mol)	FFA%
Caprylic acid (8:0)	$C_8H_{16}O_2$	144.21	0.05
Capric acid (10:0)	$C_{10}H_{20}O_2$	172.26	0.13
Margaric acid (14:0)	$C_{14}H_{28}O_2$	228.38	1.29
Palmitic acid (16:0)	$C_{16}H_{32}O_2$	256.43	30.11
Palmitoleic acid (16:1)	$C_{16}H_{30}O_2$	254.41	0.63
Stearic acid (18:0)	$C_{18}H_{36}O_2$	284.48	0.5
Oleic acid (18:1)	$C_{18}H_{34}O_2$	282.46	38.66
Linoleic acid (18:2)	$C_{18}H_{32}O_2$	280.45	9.11
Linoelaidic acid (18:2)	$C_{18}H_{32}O_2$	280.45	7.27
α-Linolenic acid (18:3)	$C_{18}H_{30}O_2$	278.44	0.95
Arachidic acid (20:0)	$C_{20}H_{40}O_2$	312.53	0.49
Lignoceric acid (24:0)	$C_{24}H_{48}O_2$	368.63	0.72
Saturated	/	/	33.29
Unsaturated	/	/	56.62
Total	**/**	**/**	**89.91**

II.4.2. Effect of reaction conditions

The results of biodiesel and glycerol obtained are shown in the figure (II.7)

A B

Figure II.7: The obtained biodiesel (A) and glycerin (B)

Biodiesel (FAME) yield was determined using the following equation below (5):

$$\textbf{Biodiesel yield} = \frac{\boldsymbol{Mass\ of\ FAME}}{\boldsymbol{Mass\ of\ WCO}} \times \mathbf{100} \ldots\ldots..(\mathbf{5})$$

II.4.2.1. Effect of transesterification with a conventional method

a. Effect of Molar ratio

We analysed the effect of the molar ratio of oil to MeOH in transforming the WCO to biodiesel with conventional method and KOH, NaOH as a catalyst. In the examination, the alcohol-to-oil ratio has been tweaked in 3:1 to18:1 by concentration of catalyst:(1% wt.), time of reaction: 120 min at 60°C.

Table II.3: Effect of ratio of oil to MeOH on transesterification with conventional method

MeOH:Oil (M) Yield (Catalyst)	03:01	06:01	09:01	12:01	15:01	18:01
Yield (NaOH)	28	71.5	64.1	57.5	51.49	50.07
Yield (KOH)	27.77	71.02	60.04	54.87	48.8	45.8

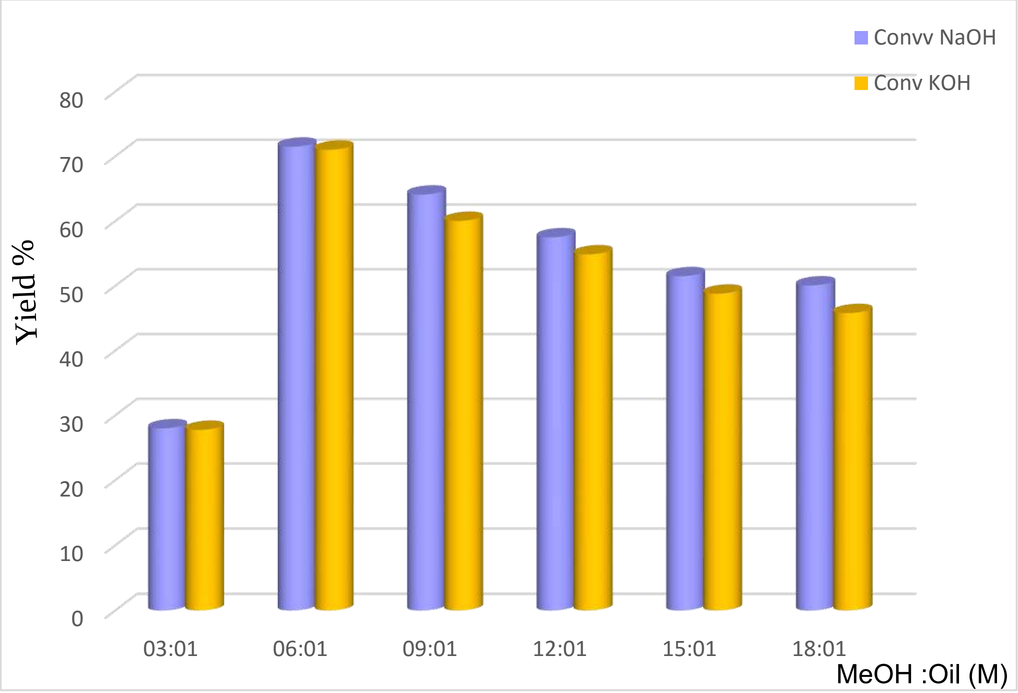

Figure II.8: Effect of ratio of oil to MeOH on transesterification with conventional method

As shown in Fig. (II.8), the result in the improvement of FAME yield was increased from 1:3 to 1:6 for both NaOH and KOH catalysts led to an improvement in the conversion ratio from 28 to 71.5% and from 27.77 to 71.02%, respectively. But the additional increase in molar ratio led to a diminution in increasing FAME yield for both two catalysts. Thus, a 1:9 molar ratio is assumed to be optimal for the transesterification of WCO. At a very increased molar ratio, the yield appears to have dropped. With a single polar hydroxyl group, extra methanol might function as an emulsifier, increasing the ester phase's solubility of glycerol, making separating more difficult. The glycerol that remains in the solution might shift the equilibrium to the left, decreasing ester conversion [21].

b. Effect of catalyst concentration

The quantity of catalyst that is utilized in the transesterification process has a massive effect on conversion [22]. This process has taken place the different values of a catalyst from 0.6 to 1.4 wt.% of NaOH and KOH) by the fixed temperature at 70°C and reaction time for 120 min with a Molar ratio of methanol to oil (6:1).

Table II.4: Effect of quantity of catalyst on transesterification with conventional method

Cat. (g) Yield (catalyst)	0.6	0.8	1	1.2	1.4
Yield (NaOH)	61.5	70.03	71.5	78.89	75.6
Yield (KOH)	59.6	69.45	71.02	78.27	73.54

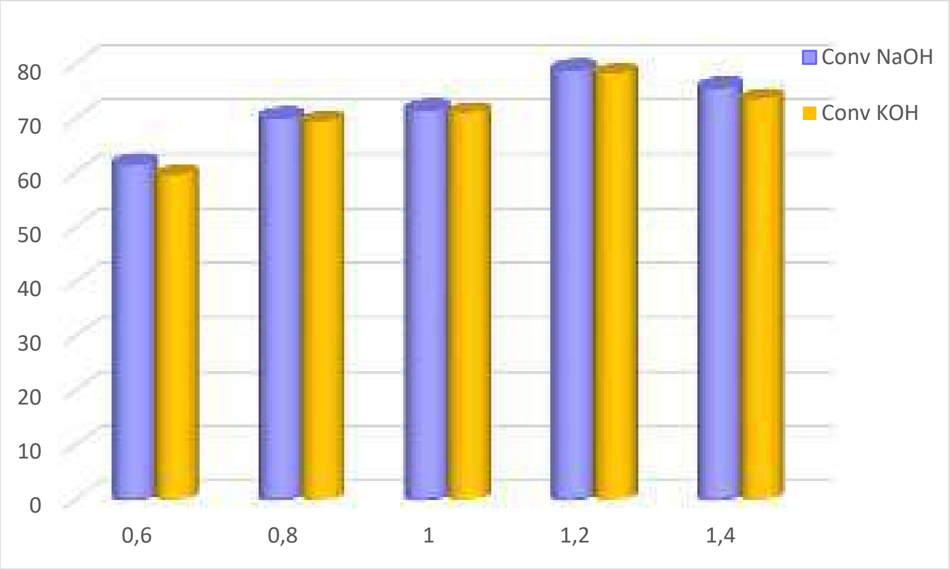

Figure.II.9: Effect of quantity of catalyst on transesterification with a conventional method

From Fig. (II.9) it was discovered that increasing the concentrations of both catalysts improved the production of biodiesel when both catalysts (NaOH and KOH) concentrations were less than 1.2% this is mostly due to saponification of the based catalyst and FAME, leading to a lower yield. The catalyst can speed up the reaction, but the high concentration amplifies the side reaction, which causes a biodiesel yield. to decrease, so the best industry production was at the amount of both catalysts 1.2% [11].

c. Effect of the reaction temperature

The temperature of the reaction is the most important parameter [22]. We initiated the effect of the reaction temperature with conventional method in different temperature in 50 to 75°C by concentration of catalyst: 1.2 % w/w and reaction time for 120 min with Molar ratio of methanol to oil (6:1).

Table II.5: Effect of the temperature on transesterification with conventional method

T (°C) Yield Catalyst	50	55	60	65	70	75
Yield (NaOH)	66.7	71	78.89	80.03	83.5	88.7
Yield (KOH)	66.84	70.05	78.27	79.8	83	88.57

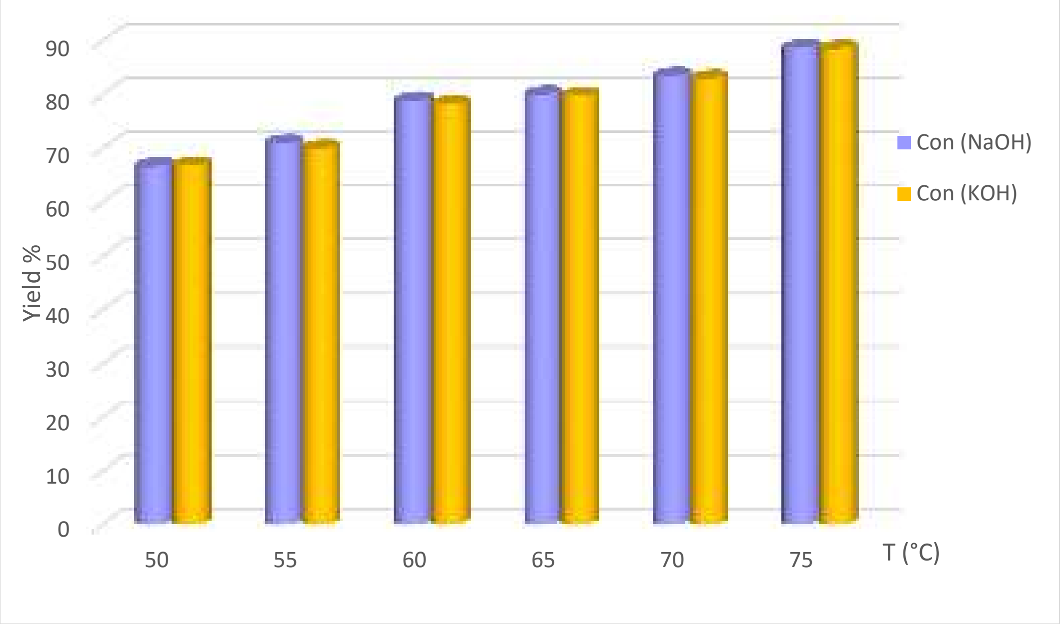

Figure II.10: Effect of the temperature on transesterification with conventional method

In figure (II.10) as shown the temperature was raised from 50 to 75°C increased the highest yield of biodiesel was achieved 88.7% ((NaOH) and 88.57% ((KOH) at 75°C. The interpretation of these results is that as the temperature increases; the biodiesel production falls because the methanol is vaporized at a higher temperature, reducing the interaction time between the reactants and the catalyst [22,23].

d. Effect of the reaction time

In the transesterification, the effect of reaction time on FAME %. We study the effect of the reaction time with conventional method. In the experiment the different time was changed from 30 to 150 min by concentration of catalyst: 1.2 % wt. reaction temperature 75°C with Molar ratio of methanol to oil (6:1).

Table II.6: Effect of the reaction time on transesterification with conventional method

t (min) Yield (catalyst)	30	60	90	120	150
Yield (NaOH)	46.87	68.7	68.7	78.89	76.45
Yield (KOH)	43	65.78	65.78	78.27	75.8

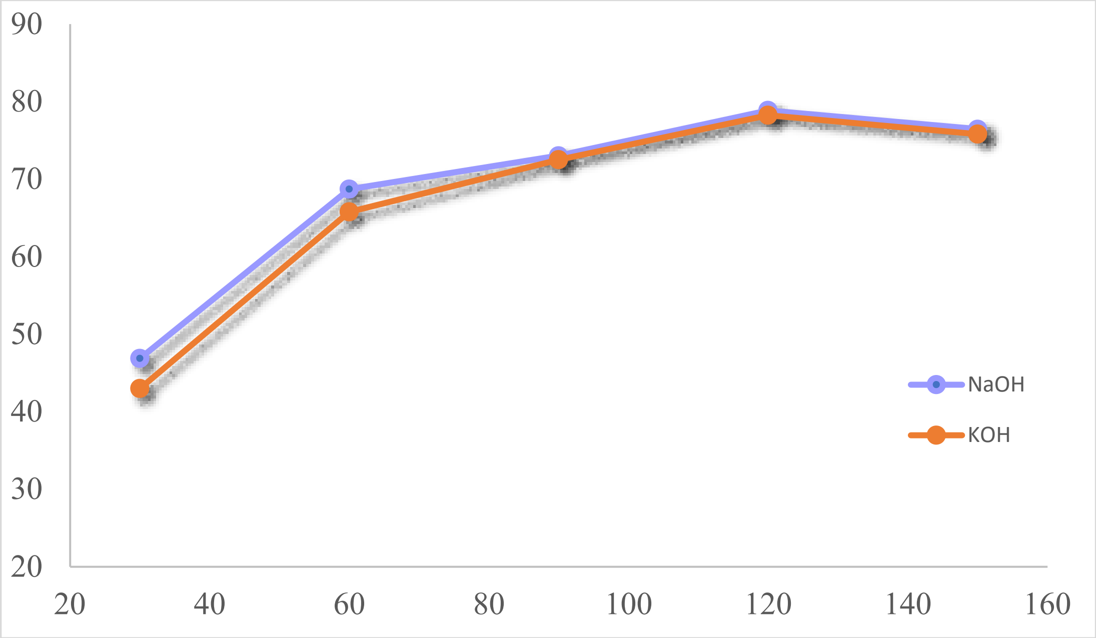

Figure II.11: Effect of the reaction time on transesterification with conventional method

Fig. (II.11) shows that the process to convert triglycerides into methyl esters had almost become complete in about 6 min of reaction time reached its maximum value 78.89% ((NaOH) and 78.27% ((KOH) in 120 minutes of reaction time by conventional method. It should be noted that the mentioned test was carried out to ensure. Lower reaction times do not allow for enough interaction of the mixtures of reactants. and increased reaction time has no effect on the conversion but favors the converse reaction (the hydrolysis of esters results in the production of glycerol)[24].

e. Results of optimization with conventional method

The results of the optimization of biodiesel production with conventional method as shown in the following table (II.8).

Table II.7: the optimization of biodiesel production with conventional method

No.	MeOH: Oil	Cat. (%wt.)	T(°C)	t (min)	R KOH (%)	R NaOH (%)
1	03:01	1	60	120	27.77	28
2	06:01	1	60	120	71.02	71.5
3	09:01	1	60	120	60.04	64.1
4	12:01	1	60	120	54.87	57.5
5	15:01	1	60	120	48.8	51.49
6	18:01	1	60	120	45.8	50.07
7	06:01	0.6	60	120	59.6	61.5
8	06:01	0.8	60	120	69.45	70.03
9	06:01	1	60	120	71.02	71.5
10	06:01	1,2	60	120	78.27	78.89
11	06:01	1,4	60	120	73.54	75.6
12	06:01	1	50	120	66.84	66.7
13	06:01	1	55	120	70.05	71
14	06:01	1	60	120	78.27	78.89
15	06:01	1	65	120	79.8	80.03
16	06:01	1	70	120	83	83.5
17	06:01	1	75	120	88.57	88.7
18	06:01	1	70	30	43	46.87
19	06:01	1	70	60	65.78	68.7
20	06:01	1	70	90	72.5	73.01
21	06:01	1	70	120	78.27	78.89
22	06:01	1	70	150	75.8	76.45

M:O: Molar ratio Methanol:oil, Cat: Catalyst, T: Temperature, t: Reaction time, Y: Biodiesel yield,

II.4.2.2 Effect of transesterification with microwave method

a. Effect of Molar ratio

The effect of the molar ratio of oil to MeOH in converting WCO to biodiesel using the microwave technique and KOH, NaOH as a catalyst is explored. The alcohol to oil ratio was changed from 3:1 to 18:1 in the experiment. by concentration of catalyst: 1% w/w, time of reaction: 6 min at 60°C. The result as show in table (II.8).

Table II.8: Effect of ratio of oil to MeOH on transesterification with Microwave method

MeOH:Oil (M) / Yield (catalyst)	03:01	06:01	09:01	12:01	15:01	18:01
Yield (NaOH)	0	76.24	83.38	80.7	77.9	76.62
Yield (KOH)	0	80.42	80.86	76.17	72.62	70.02

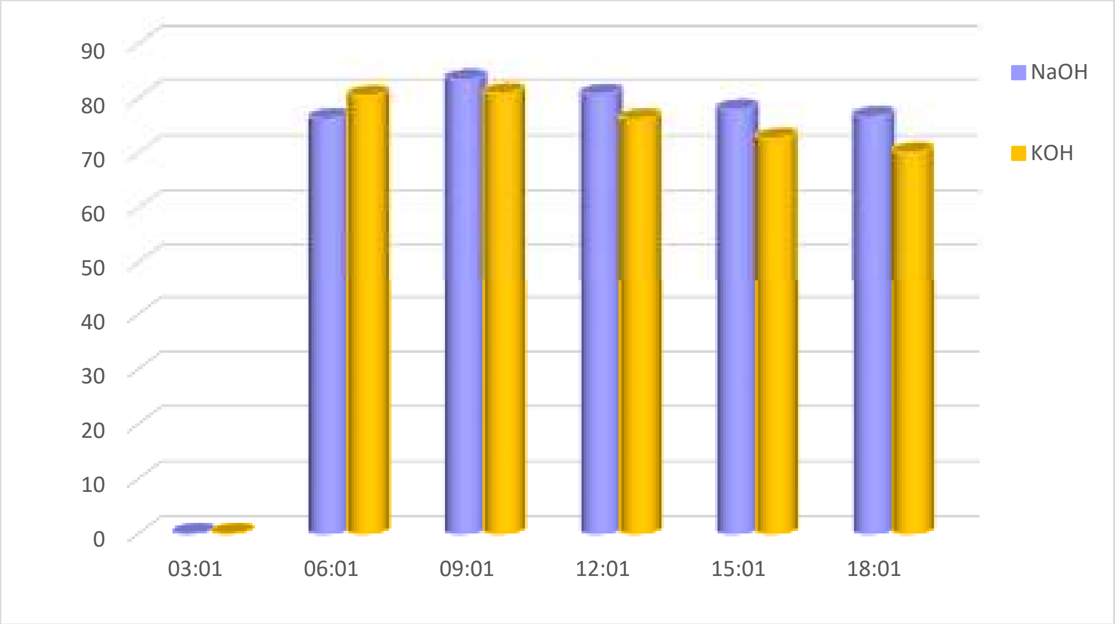

Figure II.12: Effect of ratio of oil to MeOH on transesterification with microwave method

As shown in fig (II.12) the result in the improvement of FAME yield was increased from 76.24 to 83.3% and 79.42 to 80.86% with the increment in a molar ratio from 1:6 to 1:9 for the catalyst NaOH, KOH, respectively. The additional increase in molar ratio led to a diminution in increasing FAME yield for both two catalysts. Thus, a 1:9 molar ratio is assumed to be optimal for the transesterification of WCO. At a very increased molar ratio, the yield appears to have dropped. With a single polar hydroxyl group, extra methanol might function as an emulsifier, increasing the ester phase's solubility of glycerol, making separating more difficult. The glycerol that remains in solution might shift the equilibrium to the left, decreasing ester conversion [21].

b. Effect of catalyst concentration

The amount of catalyst used in the transesterification process has a significant impact on conversion. The varying values of a catalyst (from 0.6 to 1.4 wt. percent NaOH and KOH) have been used in this procedure by the fixed a temperature at 60°C and reaction time for 6 min with Molar ratio of methanol to oil (9:1) [22].

Table II.9: Effect of amount of catalyst on transesterification with microwave method

Amount Cat. (g) Yield(catalyst)	0.6	0.8	1	1.2	1.4
Yield (NaOH)	73.86	78.78	88.38	81.58	78.23
Yield (KOH)	72.6	81.15	87.86	80.76	75.73

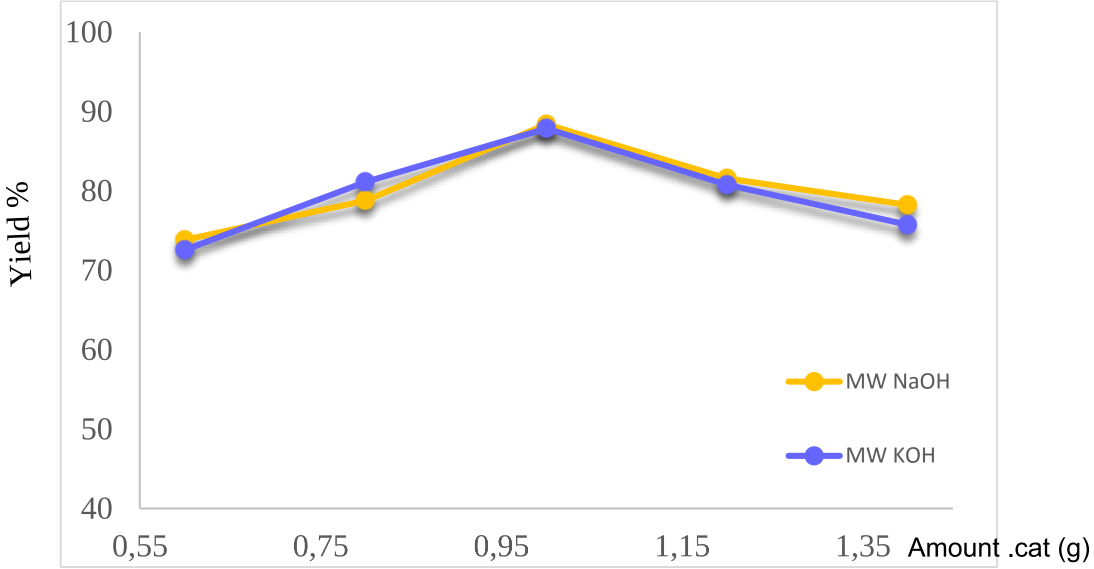

Figure II.13: Effect of quantity of catalyst on transesterification with Microwave method

Figure (II.13) shows the increase in the concentrations of both catalysts. The production of biodiesel when both catalysts (NaOH and KOH) concentrations were less than 1% this is mostly due to saponification of the based catalyst and FAME, leading to a lower yield, The catalyst can speed up the reaction, but the high concentration amplifies the side reaction, which causes a biodiesel yield. The best industry production was at the amount of both catalysts 1% [11].

c. Effect of the reaction temperature

The temperature of the reaction is the most significant parameter in a transesterification reaction [22]. With the microwave approach, we investigate the effect of reaction temperature. In the experiment the temperature was changed from 50 to 75°C by concentration of catalyst: 1% w/w and reaction time for 6 min with Molar ratio of methanol to oil (9:1).

Table II.10: Effect of the temperature on transesterification with microwave method

T (°C) Yield (catalyst)	50	55	60	65	70	75
Yield (NaOH)	80.16	87.84	88.38	89.73	94.4	90.1
Yield (KOH)	80.03	85.44	87.86	89.9	90.77	89.07

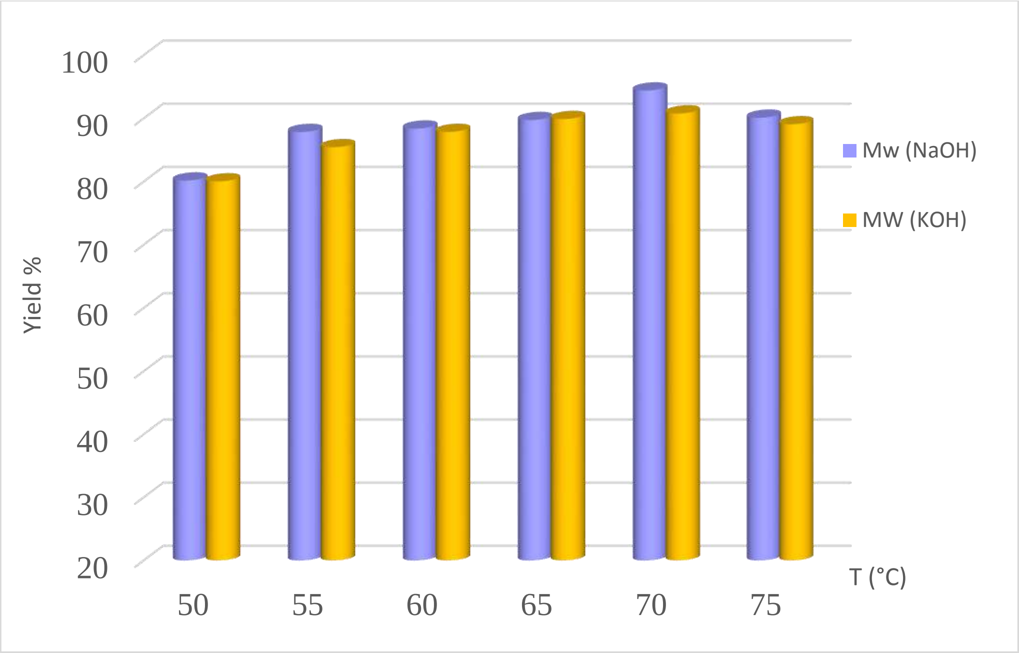

Figure II.14: Effect of the temperature on transesterification with microwave method

Figure (II.14) shows that temperature raised from 50 to 75°C and increased the yield of biodiesel from 90.84 to 85.82% with KOH but the highest yield was achieved is 94.4 at 70°C by NaOH. The interpretation of these results is that as the temperature increases; the biodiesel production falls because the methanol is vaporized at a higher temperature, reducing the interaction time between the reactants and the catalyst [22,23].

d. Effect of the reaction time

The effect of reaction time on FAME percent during transesterification. With different time intervals, we examine the effect of reaction time by concentration of catalyst: 1% w/w reaction temperature 70°C with Molar ratio of methanol to oil (9:1).

Table II.11: Effect of the reaction time on transesterification with microwave method

t (min) Yield (catalyst)	2	4	6	8	10
Yield (NaOH)	78.58	87.33	94.4	87.58	80.16
Yield (KOH)	73.2	86.46	90.77	89.72	80.11

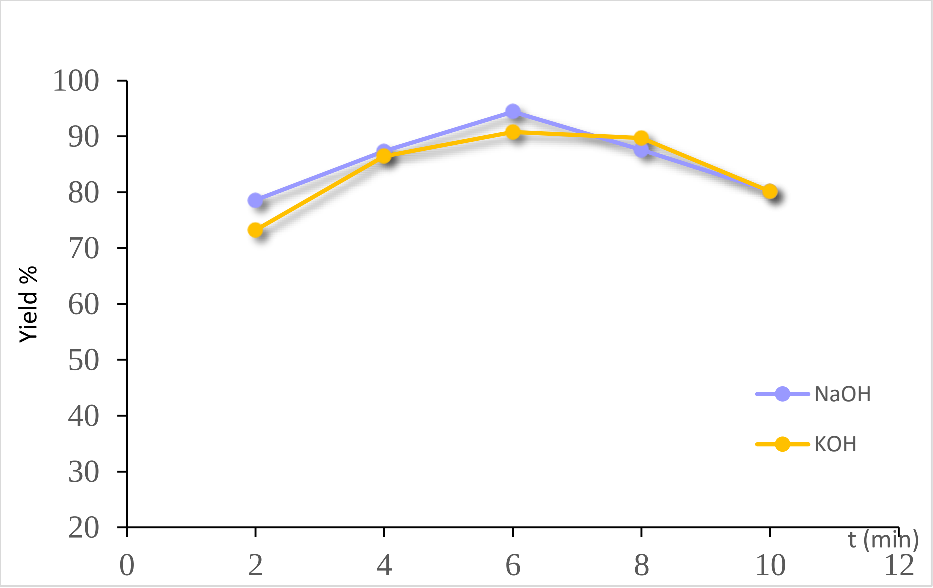

Figure II.15: Effect of the reaction time on transesterification with microwave method

Figure (II.15) shows that the process to convert triglycerides into methyl esters had almost become complete in about 6 min of reaction time reached its maximum value of 93.4%, 90.77%

with NaOH and KOH on order. It should be noted that the mentioned test was carried out to ensure. Lower reaction times do not allow for enough interaction of the mixtures of reactants. and increased reaction time has no effect on the conversion but favors the converse reaction (the hydrolysis of esters results in the production of glycerol) [24].

e. Results of optimization with microwave method

The results of the optimization of biodiesel production as shown in the following table (II.12):

Table II.12: the optimization of biodiesel production with Microwave method

No.	MeOH: Oil	Cat. (%wt.)	T(°C)	t (min)	Y KOH (%)	Y NaOH (%)
1	03 :01	1	60	6	0	0
2	06 :01	1	60	6	79.42	76.24
3	09 :01	1	60	6	80.86	83.38
4	12 :01	1	60	6	76.17	80.7
5	15 :01	1	60	6	72,62	77,9
6	18 :01	1	60	6	70.02	76.62
7	09 :01	0.6	60	6	72.6	73.86
8	09 :01	0.8	60	6	81.15	78.78
9	09 :01	1	60	6	87.86	88.38
10	09 :01	1.2	60	6	80.76	81.58
11	09 :01	1.4	60	6	75.73	78.23
12	09 :01	1	50	6	80.03	80.16
13	09 :01	1	55	6	85.44	87.84
14	09 :01	1	60	6	87.86	88.38
15	09 :01	1	65	6	89.9	89.73
16	09 :01	1	70	6	90.77	94.4
17	09 :01	1	75	6	89.07	90.1
18	09 :01	1	70	2	73.2	78.58
19	09 :01	1	70	4	86.46	87.33
20	09 :01	1	70	8	89.72	87.58
21	09 :01	1	70	10	80.11	80.16

M:O: Molar ratio Methanol:oil, Cat: Catalyst, T: Temperature, t: Reaction time, Y: Biodiesel yield

II.4.3. Comparison of the effect of different methods

The effect of different methods on biodiesel production was studied, where we applied three methods of transesterification of used cooking oil using optimum conditions for the transesterification reaction obtained in this study. and the table (II.13) as shows the comparison of the yield of biodiesel production by several methods of transesterification.

From table (II.13), it can be observed that ultrasonic and microwave method can increase the biodiesel yield or conversion by 15- 21% in comparison to conventional method. In short, it can be concluded that ultrasonication can achieve higher biodiesel conversion or yield than conventional stirring under comparable reaction conditions.

An explanation of these results, in transesterification reaction the ultrasonic method causes bubble cavitations near the phase boundary between the alcohol and oil phases, thus producing large amount of micro bubbles, creating intimate mixing of the immiscible reactants near the phase boundary and thus induce emulsification [25,26]. With the formation of such emulsion, to reduce mass transfer resistance between the alcohol and oil phases increases, thus the chemical reaction kinetics will become faster [27].

Table II.13: Comparisons of the effect of different methods on biodiesel production

Optimum reaction condition	**Conventional**	**Microwave**	**Ultrasonic**
Catalyst	NaOH	NaOH	NaOH
Oil WCO (ml)	100	100	100
Alcohol	Methanol	Methanol	Methanol
Molar ratio of oil to methanol (M)	1:6	1:9	1:9
Catalyst loading (wt%)	1%	1%	1%
Temperature (°C)	70	70	70
Time (min)	120	6	30
Yield of biodiesel (%)	88.7	94.4	99.7

II.4.4. Effects of two-step transesterification

The effect of pretreatment of WCO oil with sulfuric acid was investigated to the optimization of transesterification reaction conditions are essential to obtain the highest possible output of fatty acid methyl ester (FAME) in biodiesel synthesis.

The results in table (II.14) shows that the highest concentration of fuel was with the pre-treated oil acid, where the best yield was 96.64% by NaOH a catalyst. The interpretation of these results, waste cooking oil includes a lot of free fatty acids, in such a case, a part in the operation may go through saponification, resulting in the formation of soaps, this decreases biodiesel yield and has an impact on product quality. But when acid catalysts are used for esterification, the free fatty acid (FFA) content of the oil is effectively reduced, and the modified oil is now available for alkali catalysis. [28,29].

Table II.14: Comparisons of biodiesel yield obtained with or without pretreatment in this study the optimum transesterification process conditions

Optimum reaction condition				
Catalyst	KOH	KOH	NaOH	NaOH
Oil feedstock	WCO	WCO	WCO	WCO
Alcohol	Methanol	Methanol	Methanol	Methanol
Pretreatment with H_2SO_4	-	1:6/ 1%	-	1:6/1%
Molar ratio of oil to methanol (M)	1:9	1:9	1:9	1:9
Catalyst loading (wt.%)	1%	1%	1%	1%
Temperature (°C)	70	70	70	70
Time (min)	6	8	6	8
Yield of biodiesel (%)	90.77	92.47	94.4	96.64

II.4.5. Comparison with previous studies

In order to compare the biodiesel conversion or yield attained between our study and previous studies, there are ample of studies which had been published on the transesterification by utilizing non-edible feedstocks. Comparison of biodiesel yield using:

❖ Microwave method

Microwave assisted transesterification is more energy-efficient owing to the direct transfer of energy into interior molecules of the reactants by microwave radiation, keeping other conditions unchanged, microwave radiation can reduce the reaction time from several hours to several minutes, which eliminates the need for preheating. in which the results to compare the biodiesel production using Microwave method between our study and previous studies are tabulated in table (II.15).

Table II.15: Comparisons the biodiesel yield between our study and previous studies by microwave method

Biomass	Optimum reaction condition	R %	Ref.
WCO	KOH : 1%(w/v), M=8:1, T= / (°C), t=6 (min), P= 500 (W)	96.8	[30]
WCO	NaOH : 1%(w/v), M= 6:1, T=60 (°C), t= 5 (min), P= 500 (W)	98.87	[31]
WCO	NaOH : 2 %(w/v), M=5:1, T=65 (°C), t= 30 (min), P=250 (W)	94.6	[32]
WCO	NaOH: 0.8%(w/v), 12:1, 65 (°C), 2 (min), P= 600 (W)	98.2	[33]
WCO	NaOH : 1%(w/v), M= 9:1, T=70 (°C), t= 2 (min), P= 300 (W)	94.4	This study

The table (II.15) shows that the reaction conditions applied in our study provided the less productivity compared to the previous studies where it reached 94.4%. Azcan N. et al. [31] and Hsiao M.C. et al, [33] note that the increase in energy (500 W) leads to good interaction and very high productivity 98.8% 98.2% in the short reaction time (2-5 min). From comparing the study of Hong I.K. et al. [30]with Azcan N. et al. [31] we note that the catalyst that provides the best productivity (98.87%) is NaOH compared to KOH in similar conditions. Supraja K.V. et al. [32], showed that the main factors that affect the rate of the reaction are the energy, in spite of doubling the amount of the stimulus, it gave less return than a study of Hsiao M.C. et al. [33]. As for our study, we showed that raising the reaction temperature does not affect the increase in biodiesel production, as the production rate reached 94.4% at 70 °C, in contrast to a study that reached 98.86% at 60 °C.

- **Ultrasound-assisted method**

Overall, the effect of ultrasonication is advantageous to be used for process intensification in biodiesel production and high conversion. They are normally inexpensive and have low reaction conditions, in which the results to compare the biodiesel conversion or yield attained between our study and previous studies are tabulated in table (II.16).

Table II.16: Comparisons the biodiesel yield between our study and previous studies by ultrasound method

Biomass	Optimum reaction condition	R %	Ref.
WCO	KOH :1%(w/v), M=12:1, T= 40 (°C),t= 30 (min), P= 450 (W)	90	[34]
WCO	KOH: 1%(W/V), M= 6:1, t=8 (min), P=240 (w)	80	[35]
WCO	KOH : 1%(w/v), M=7:1, T= 50 (°C), t=60 (min), P= 500 (W)	99	[36]
WCO	KOH : 1%(w/v), M= 7:1, T=50 (°C), t= 60 (min), P=31 (W)	97.1	[37]
WCO	NaOH : 1.25%(w/v), M=9:1, t= 2 (min), P=150 (W)	96.8	[38]
WCO	NaOH: 1%(w/v), 5:1, 50 (°C), 60 (min), 500 (W)	99	[36]
WCO	NaOH : 1%(w/v), M= 9:1, T=70 (°C), t= 30 (min), P=50 (W)	99.7	This study

The table (II.16) shows that the reaction conditions applied in our study provided the best productivity compared to the previous studies where it reached 99.7%. Khosravi E. et al. [36] note that the increase in energy (500w) and the reaction time is long (60 min) leads to good interaction and very high productivity 99%. However, the reaction temperature is low (50°C). The results of both Aghbashlo M. et al. [37] and Martinez-Guerra E. et al. [38] showed that the main factors that affect the rate of the reaction are the energy and the duration of the reaction, where have an inverse relationship when the energy is raised to (150 W) and reducing the reaction time (2 min) it gets the productivity of 96.8% but when the power is reduced to (31 W) and increasing the reaction time (60 min) to lead to production 97.8% from biodiesel. A study by Maddikeri G.L. et al. [34] showed that temperature is an important factor, as productivity decreased to 90% by keeping the reaction temperature below (50°C) Although the energy is (450 W). As for our study, we note that the reaction temperature gives the best production (99.7%) is at the boiling point of methanol (70°C) less card (50 W) Good reaction time (30 min)

- **Different catalysts**

The biodiesel yields at optimum conditions by using different catalysts are tabulated in Table (II.17). The highest biodiesel yield (99.7%) was achieved by using Sodium hydroxide (NaOH) while the lowest yield (84%) was achieved by using tripotassium phosphate (K_3PO_4).

Table II.17: Comparisons the biodiesel yield between our study and previous studies by using different catalysts

Biomss	Optimum reaction condition	R %	Ref.
WCO	K_3PO_4:3%(w/v), M=6:1, T= 60 (°C),t= 120 (min), P= 375 (W)	92	[34]
WCO	CaDG: 1%(W/V), M= 9:1, T=60°C, t=40 (min), P=120 (W)	93.5	[39]
WCO	CaO : 1%(w/v), M=7:1, T= 50 (°C), t=60 (min), P= 500 (W)	92.5	[40]
WCO	KOH : 1.25 %(w/v), M=7:1, t= 1 (min), P=240 (W)	99	[41]
WCO	NaOH : 1%(w/v), M= 9:1, T=70 (°C), t= 30 (min), P=50 (W)	99.7	This study

II.4.6. Results of product quality analysis

II.4.6.1. Results of FTIR Analysis

Infrared spectroscopy was used to monitor the transesterification reaction and to ensure the success of the reaction, (Fig.II.16) and (Table II.18) shows the most important changes that occurred on the absorption spectrum of the sample before esterification (A) and after (B).

Table II.18: the absorption of functional groups in the IR spectrum of WCO compared to biodiesel

Waste cooking oil		Biodiesel	
(CH_2-O-)	1157 cm^{-1}	(CH3-O-)	1194 cm^{-1}
C=O	1743 cm^{-1}	C=O	1741 cm^{-1}
(CO-O-O)	1463 cm^{-1}	(CO-O-O)	1459 cm^{-1}
/	/	(CO-O-CH_3)	1435 cm^{-1}
Absorption of stretching vibrations for CH, CH_2, CH_3 appear at 3008.372 cm^{-1}, 2922.269 cm^{-1} and 2852.712 cm^{-1}			
Absorption of stretching vibrations for CH_2, CH_3 appear at 1463.109 cm^{-1} 1160.223 cm^{-1}			
Absorption at 721.865 cm^{-1} is indicate on methylene functional groups (-$(CH_2)_n$-)			

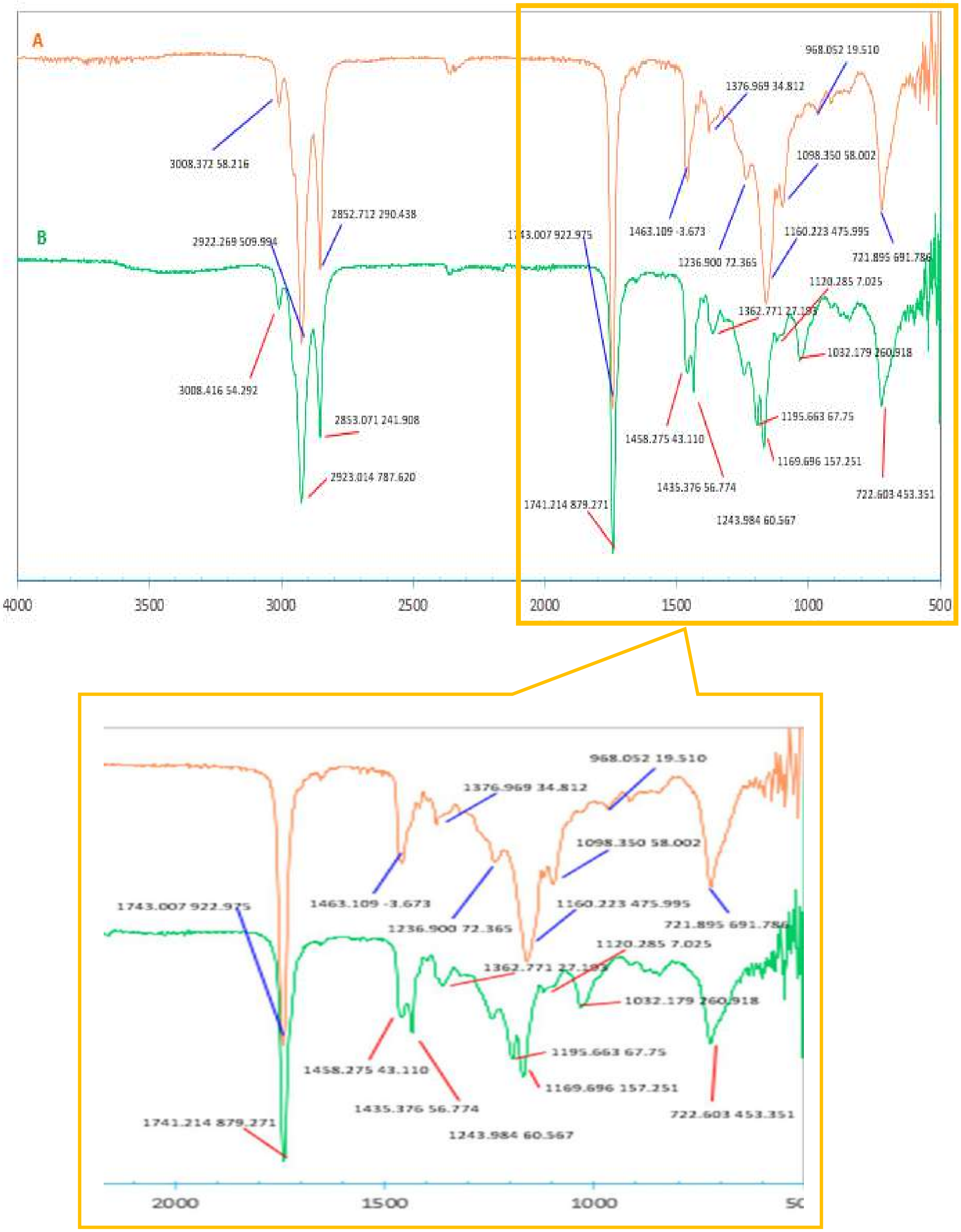

Figure II.16: Fourier Transform Infrared Spectroscopy (FTIR) spectrum of WCO (A) and Biodiesel (B)

II.4.6.2. Results of GC-MS Analysis

The results of analysis by GC-MS led to the identifying of twenty-five (25) of GC compounds were identified using mass spectrometry in conjunction with analysis by GC-MS (NIST 17 MS Library). Figure (II.19) reflects a typical GC-MS chromatogram and showed the main peaks and the composition of the analysis is shown in Table (II.19) represents the retention times that are relevant to the name, molecular formula, and structure of each peak refers to one FAME content, and the overall content is represented by the peak area and details of the compounds.

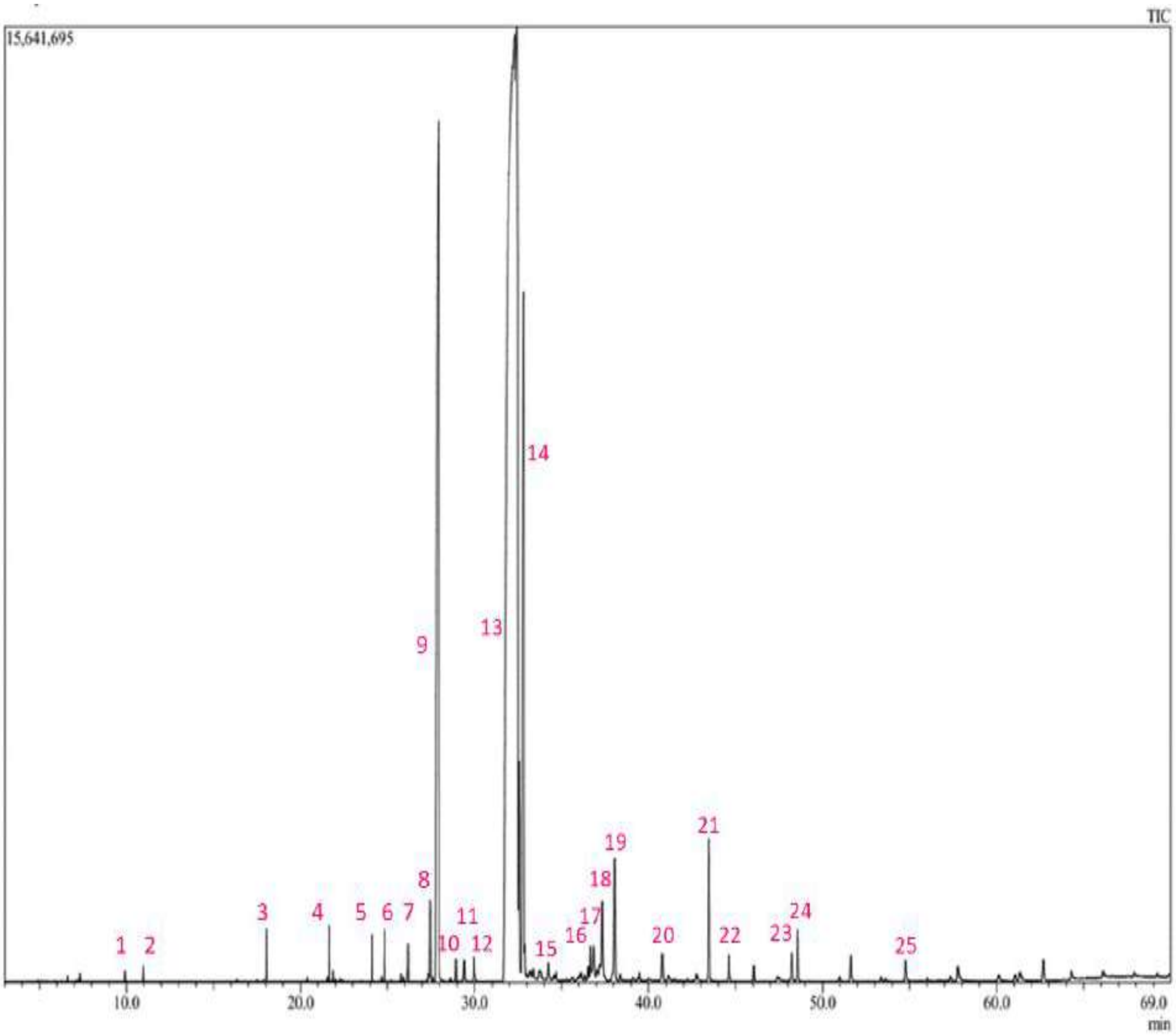

Figure II.17: GC-MS chromatogram of biodiesel

. **Table II.19:** The chemical composition of synthesized biodiesel by GC-MS

Peak	Retention time	Name
1	9.932	Octanoic acid, methyl ester
2	10.974	Nonanoic acid, 9-oxo-, methyl ester
3	18.049	Tridecanoic acid, 12-methyl-, methyl ester
4	21.645	7,10-Hexadecadienoic acid, methyl ester
5	24.110	9-Hexadecenoic acid, methyl ester, (Z)-
6	24.821	6-Octadecenoic acid, methyl ester, (Z)-
7	26.190	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
8	27.441	Hexadecanoic acid, methyl ester
9	27.944	9,12,15-Octadecatrienoic acid, methyl ester
10	28.933	Hexadecanoic acid, 15-methyl-, methyl ester
11	29.405	(Z)-10-heptadecenoic acid methyl ester
12	29.967	17-Octadecynoic acid, methyl ester
13	32.265	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
14	32.309	8,11-Octadecadienoic acid, methyl ester
15	34.236	6-Octadecenoic acid, methyl ester, (Z)-
16	36.654	Heptadecanoic acid, 16-methyl-, methyl ester
17	36.842	Propionic acid, 3-benzoylamino-3-(4-ethoxyphenyl)-, ethyl ester
18	37.332	11-Eicosenoic acid, methyl ester
19	38.054	Eicosanoic acid, methyl ester
20	42.764	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
21	43.458	Docosanoic acid, methyl ester
22	44.616	cis-11,14,17-eicosatrienoic acid methyl ester
23	46.045	Tricosanoic acid, methyl ester
24	48.571	Tetracosanoic acid, methyl ester
25	54.773	methyl cis-15-tetracosenoate

II.4.6.3. Results of physicochemical properties

To appraise the biodiesel producer's quality as compared to the international standards were studied five of the most important characteristics of WCO, biodiesel, and Petro-diesel were assessed, which were comparable of Petro-diesel (Table.II.20) that the table 3 shows that biodiesel met standards [7].

Table II.20: Comparison of biodiesel produced with international standards

Properties	WCO	Biodiesel	Petro-diesel	ASTM B100
Density	919	877	834–860	860 to 900
Viscosity (20 °C, 40°C, $mm^2.s^{-1}$)	76.72, 75.05	5.97, 4.18	1.9 to 3.8	1.9 to 6.0
Acid value (mg KOH/g sample)	0.9	0.5	0.5	0.5
Saponification value (mg KOH/g)	213	90.18	-	-
Flash point (°C)	250	140	60–70	100–170
Pour point (°C)	/	-16	−35 to −15	−15 to −16
Cloud point (°C)	/	-10	−15 to −5	−3 to−12
Cetane number	/	50	46	47 minimum

II.5. Conclusion:

Finally, the results of this study showed that low-cost and high-quality fuel prepared by waste cooking oil pretreated with ultrasonic method has percentage the yield of biodiesel was 99.7% and this high biodiesel yield concludes of the waste oil that using NaOH as a catalyst. The optimum conditions for maximum yield in this study with the microwave method are 1 wt.% catalyst concentration, 1:09 oil to methanol molar ratio, 8 min reaction time at 70°C, and 800 rpm. The groups of functional and FTIR and MS spectroscopy are used to validate conversion efficiency. Furthermore, the physicochemical characteristics of WCO and biodiesel demonstrated biodiesel's potential as a diesel fuel replacement.

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Chapter III

Bioethanol preparation methods

III.1. Introduction

The bioethanol is nowadays increasingly used as ecofriendly is one of the most important biofuels generated from renewable resources such as sugarcane in Brazil, corn in the US, and wheat and sugar beet in the European Union (EU) [1]. Bioethanol is ethanol extracted by fermentation, fermentation is anaerobic bioconversion in the presence of yeast and inappropriate conditions of temperature and pH, the fermentation of sugars contained in the biomass [2,3]. Vegetable biomass as feedstock for bioethanol production allows for recycling the CO_2 released during combustion, reducing the CO_2 emissions [3,4]

In this part of study, valorization of biomass is made possible for any product with low market value obtaining bioethanol which constitutes a product of high added value, and that from the bio-valuation of poor-quality dates in Algeria.

III.2. Experimental plan

III.2.1. Sample preparation

III.2.1.1. Sample collection

We took the samples from the city of Ouargla, a region characterized by the production of a large quantity of dates, these samples were collected in 2018 at the Babziz farm in Hassi Ben Abdallah, we chose to study the species of: Degla Bayda.

III.2.1.2. Sample preparation

The samples were cleaned with tap water and pitted. The cores were placed in the shade to dry them within a week, finally crushed by a grinder (Figure III.1).

Figure III. 1: Samples of Degla Bayda

The general diagram adopted for the realization of this study is summarized by the figure (III.2).

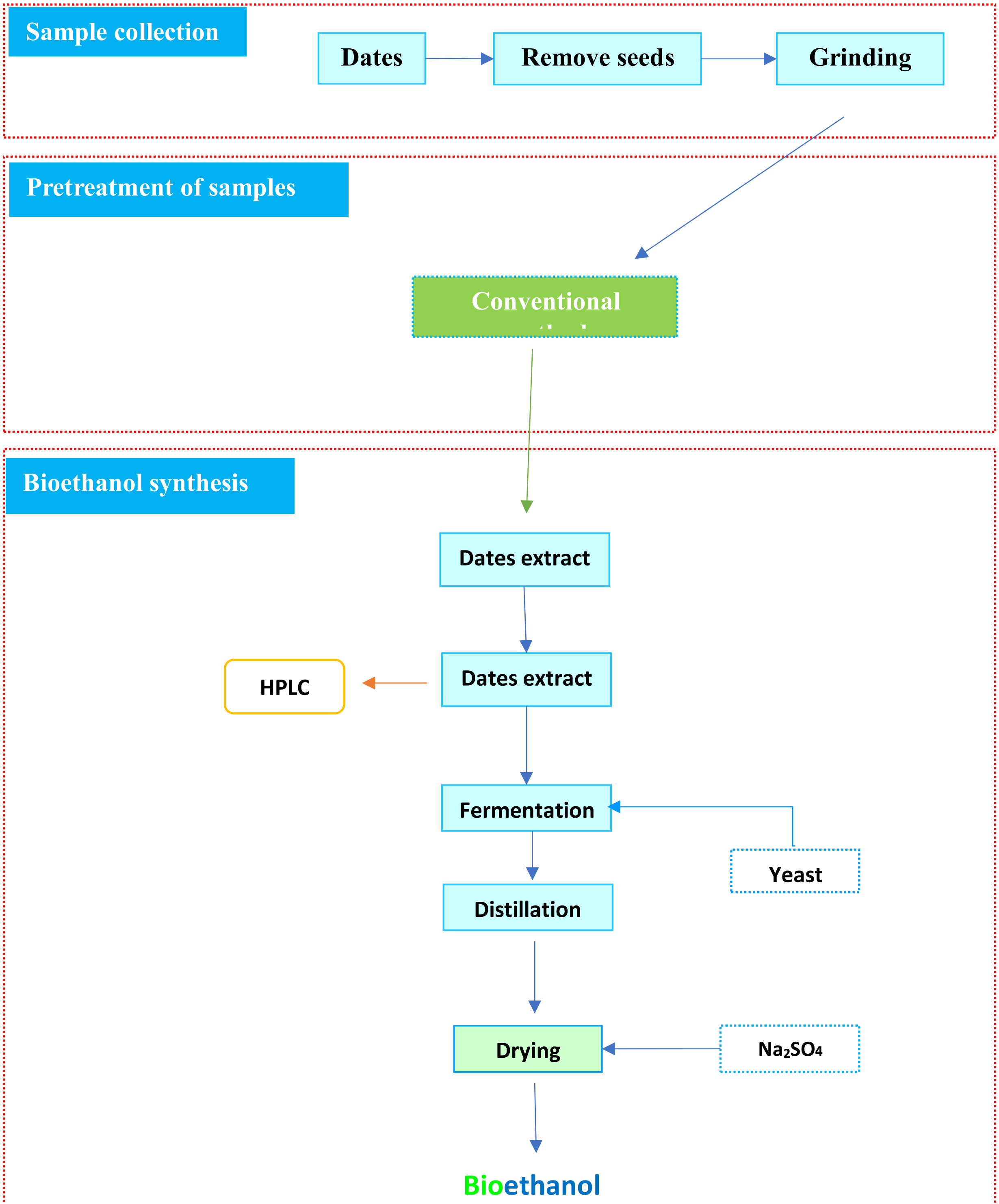

Figure III. 2: Scheme of bioethanol synthesis

III.3. Fermentation

Alcoholic fermentation is the most widely used and least expensive conventional technology for bioethanol production.

In our study, we applied this technique to date products with low market value, which is white sugar, by using bread yeast (Fig. III.3) to obtain bioethanol. A conventional method (soaking in hot water) was also used for the pretreatment of date samples.

The effect of changing the reaction conditions on the fermentation reaction and improving productivity will also be studied. This study will be done in the (conventional method) way.

The conditions under which the study will be conducted are as follows:

- ✓ The effect of pH on the reaction.
- ✓ The effect of temperature on the reaction.
- ✓ The effect of the amount of yeast on the reaction.
- ✓ The effect of reaction time on reaction.

Figure III.3: Type of yeast used

III.3.1. Bioethanol synthesis protocol

III.3.1.1. Extraction of date juice

The juice is a sweet liquid that is prepared, the juice is obtained by maceration of dates pitted in hot water for 5 hours. The quantity is determined by 400 g of the date on each 1L of distilled water. Finally, the solution is filtered to separate the musts and dates, we get 400 ml of date juice [5,6].

III.3.1.2. Fermentation

Ethanol fermentation was carried out using 100 ml of date juice which is the best hydrolysis treatment based on the number of sugars produced from date. yeast is used *Saccharomyces cerevisiae* in the fermentation process by different the amount under various conditions of pH and temperature and time for the best yield of bioethanol production· [7].

III.3.1.3. Distillation and rectification

At the end of the fermentation, a date wine is obtained which must be filtered to separate the yeast. Fractional distillation is a process used to separate ethanol from water according to their different volatilities. This process essentially consists of boiling the ethanol-water mixture. It is distilled at a temperature of about 80°C and 78°C [2,6].

- **Bioethanol Yield:**

We calculated the yield of bioethanol extracted using the following relation:

$$\boldsymbol{Y = \frac{Volume\ of\ Alcohol\ (ml))}{Quantity\ of\ Date\ (Kg)} \times 100 \ldots\ldots\ldots..(1)}$$

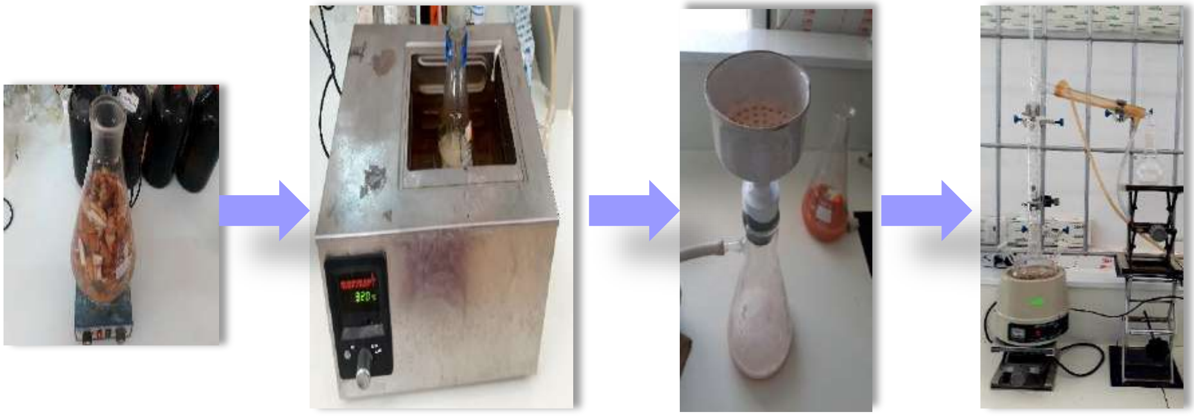

Figure III.4: Bioethanol preparation method

III.3.2. Analysis of date juice

All the analyzes of physical characterization of the date were carried.

III.3.2.1. Determination of the water content

The water content is determined in an oven at 105°C. by drying 10 g of dates or juice for 18 hours. Remove the sample from the oven, place them in the desiccator, and after cooling, weigh them. The operation is repeated until a constant weight is obtained. The water content is equal to the loss of mass undergone under the conditions of the measurement [8].

$$\boldsymbol{H\%} = \frac{\boldsymbol{Mi - Mf}}{\boldsymbol{Mi}} \times \boldsymbol{100} \ldots\ldots\ldots.(\boldsymbol{2})$$

Where:

H%: Water or humidity content;

M_i: Initial mass “before drying”

M_f: Final mass "after drying"

III.3.2.2. Carbohydrate analysis

Determination of sugar in date by HPLC was carried out according to the method described by Alghamdi B.A. et al. [9].

- **Preparation of solution**
  - **The first solution:** We prepared a standard sugar solution using the following standard solutions: fructose, glucose, and sucrose at a concentration of (2g/l), the working sugar mixture solution was prepared by transferring 1 ml of each standard solution of the three sugars to 10 ml volumetric flask and then the final volume was completed with distilled water.
  - **The second solution:** Sample preparation was carried out from the date sample by dissolving 2.5 g of sample in 25 ml distilled water in a beaker. The resultant solution was filtered by a filter of 0.45 µm.

- **Operating conditions**

High-performance liquid chromatography analysis was performed on a Shimadzu apparatus. The operating conditions are collated in Table (III.1).

Table III.1: Operating conditions for HPLC analysis.

HPLC Program	
Capillary column	ZORBEX carbohydrate 150×4.6 mm
Mobile phase	20:80 v/v distilled water/ acetonitrile
Temperature of column	27°C
Injected quantity	10 µl (1.5 ml/min)
Detector	LC-RID

Figure III.5: HPLC apparatus (Shimadzu)

III.3.2.3. Reducing sugars analysis

Determination of reducing sugar in date by UV–vis was carried out according to the method described by Başkan K.S. et al. [10].

- **Reagent and standard solutions**

Were prepared the following standard solutions:

1. DNS solution 1% (w/v)
2. Sodium potassium tartrate solution 40% (w/v)
3. Phenol solution 0.2% (w/v)
4. Potassium disulfite solution 0.5% (w/v)
5. NaOH solution1.5% (w/v)

- **Preparation of DNS Reagent:** DNS, sodium potassium tartrate, phenol and potassium disulfite solutions were mixed at 1:1:1:1 (v/v/v/v) ratio.
- **Glucose standard solution:** we prepare a standard solution of glucose with a concentration of (0.1 mM), and from it is prepared different concentrations (0.01-0.1 mM).

- **Operation:**

Added 100 µl of DNS reagent to 1ml of each glucose standard solution, and the mixture was vortexed and incubated in a boiling water bath for 5 min. After cooling in the ice bath, the absorbance of the sample was recorded at 540 nm. The test tube contained the same volume of solvent (water) in place of glucose standard, The measure of maximum absorption of the DNS. In the same way, the absorption of date extract is measured. To calculate the percentage of reducing sugars, we applied the relationship (3). This study was carried out using a (UV-Vis Cary300 (Agilent)) device.

$$C\,(mg/100g) = \left(\frac{A}{K} \times F \times \frac{V}{P}\right) \times 100 \ldots\ldots\ldots..(3)$$

whereas:

C: Amount of reducing sugars (mg/100g).

A: Optical absorbance of the extract at 540 nm.

K: Slope of the standard curve.

F: Extension factor for the extracts.

V: Volume in dissolved sample (ml).

P: Initial mass of the dry sample in grams

Figure III.6: UV-vis Cary300 (Agitant)) apparatus

III.3.3. Bioethanol analysis

III.3.3.1. Bioethanol characterisation

To assess the bioethanol produced quality and compare it to the universal standards, table (III.2) shows five of the biodiesel characteristics were measured [11,12].

Table III.2: Characteristics of the bioethanol obtained

Property	Equipment	Test standard
Density	Anton Parr DMA 3001	ASTM D1298
Kinematic viscosity	Anton Parr DMA 3001	ASTM D445
Boiling Point	/	/
Degree of ethanol	/	/
FTIR	Agilent Resolutions Pro	ASTM D6079

III.4. Results and Discussion:

III.4.1. Physical characterization of the date

The physico-chemical properties of date extract were determined and the results are shown in the table (III.3).

Table III.3: Physico-chemical characteristics of date

Characteristics	
Date weight (g)	05,25±0,46
Pulp weight (g)	04,55±0,59
Seed weight (g)	00,98±0,08
Seed / date ratio (%)	18,67±0,17
Pulp / date ratio (%)	86,67±1,28
Humidity (%)	14,55±1,54
pH	5,4±0,45

III.4.2. Results of carbohydrate analysis

The determination of different sugar contents in date juice. One of the major characteristics of date is that fructose is present in a higher amount than glucose. Approximately, about 50% is fructose and 40% glucose as shown in the figure (III.7), the sucrose is also present in low amounts ideally not more than 0.2%. The total sugars in samples were analyzed and have been found in the range of 50.26–74.74 g/100 g of date. The chromatographic results (Table III.4) showed the presence of the sugars like fructose and glucose in data samples. Total sugar content was highest in the sample with a ratio (80 %).

Table III.4: Total sugar components in date samples as determined by HPLC

Sample	Sugar content (%)			
	Fructose	Glucose	Sucrose	Total
Date	50%	40%	0.2%	80 %

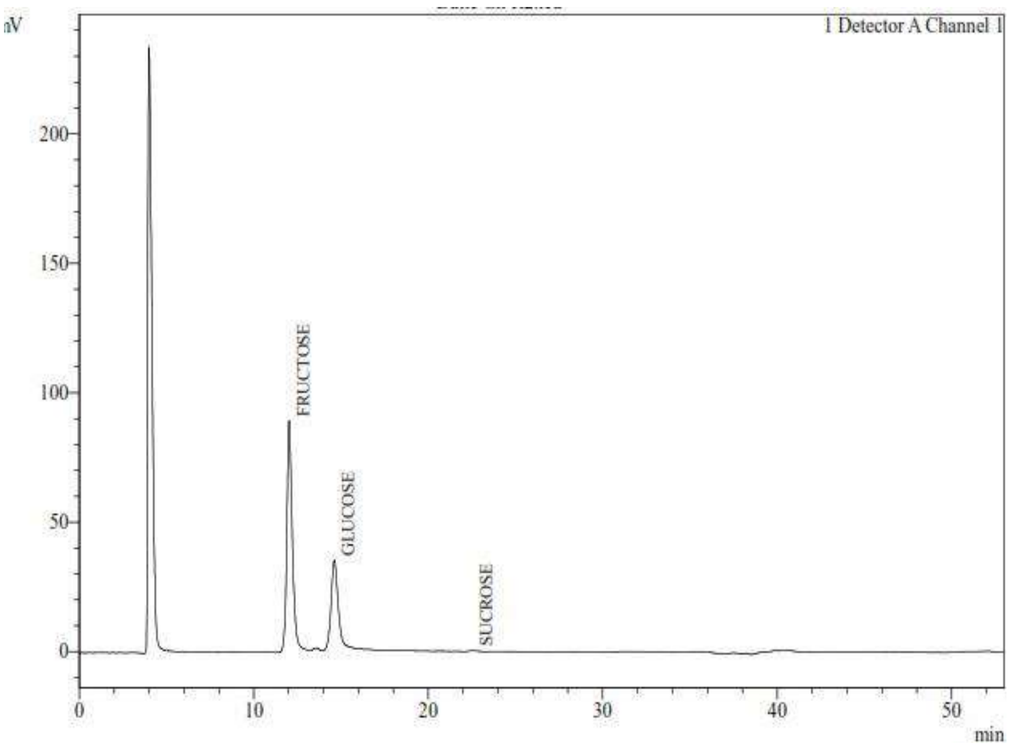

Figure III.7: Representative HPLC chromatogram of fructose, glucose and sucrose

III.4.3. Results of reducing sugars analysis

The determination of reducing sugar contents in date juice we prepared the calibration range as shown in table (III.5). The reducing sugars in samples were analyzed and have been found in the ratio of 49% in date.

Table III.5: Glucose standard range for the determination of reducing sugars

Glucose solution (ml)	0	0.5	1	1.5	2
Distilled water (ml)	10	9.5	9	8.5	8
Volume to be withdrawn (µl)	100	100	100	100	100
Reagent of DNS (µl)	100	100	100	100	100
Concentration of sugars (µg /µl)	0	0.5	1	1.5	2

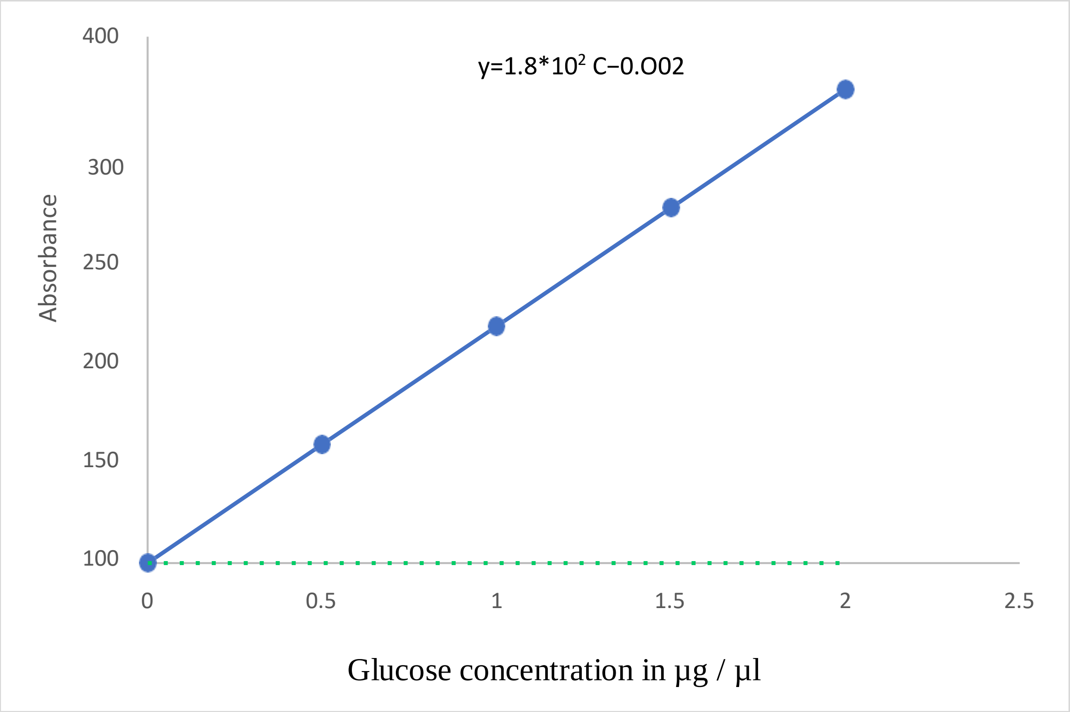

Figure III.8: Glucose standard range for the determination of reducing sugars

III.4.4. Effect of reaction conditions on bioethanol yield

III.4.4.1. Effect of pH

The effect of pH on ethanol fermentation was studied at different pH ranges from pH 4.0 to 6.0 for used yeast strain namely commercial *Saccharomyces cerevisiae*, incubation was carried out at 32 °C, for 24 hours of the fermentation period. Glucose conversion was continued simultaneously dur in the fermentation. As shown in (Fig. III.9) the yield of ethanol was increased between pH 4.0 to 5.0 and then decreased below this value. The maximum of ethanol yield 36.25% at pH 5.0.

Table III.6: Effect of pH on bioethanol yield

pH	4	4.5	5	5.5	6
Bioethanol yield (%)	18.75	25	36.25	21.25	17.5

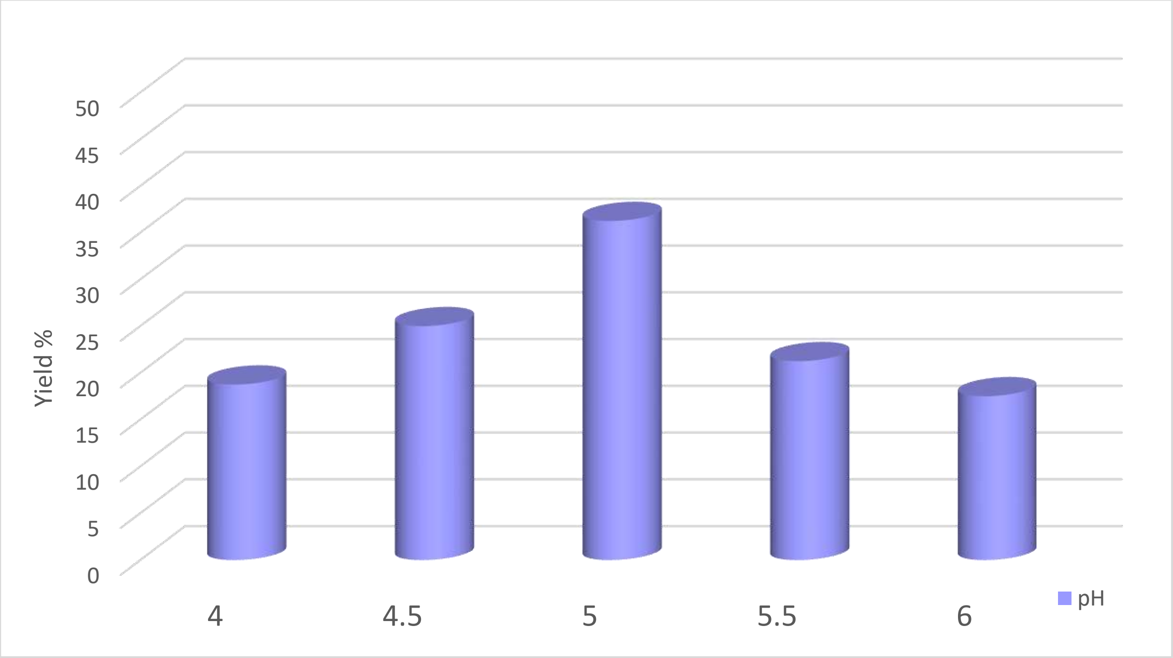

Figure III.9: Effect of pH on bioethanol yield

III.4.4.2. Effect of temperature

Bioethanol production affected by the temperature variation. Under suggested fermentation protocol, commercial *Saccharomyces cerevisiae* yeast strain showed diverse results under different temperatureconditions (28-36 °C) with regarding to bioethanol production (fig. III.10).

The best bioethanol yield was reported 36.25 % at 32 °C, while at 30 °C and 34 °C, bioethanol productions were 18.25% and 23.75% on the order, a reduction in ethanol production was occurred at 28 °C and 36°C was by 15.5%, 17.5% on the order. These results may be due to the reduction in the growth of *Saccharomyces cerevisiae* yeast at higher and very low temperature that too decreased the rate of fermentation process [13].

Table III.7: Effect of Temperature on Bioethanol Yield

Temperature (°C)	28	30	32	36
Bioethanol yield (%)	15.5	18.25	36.25	17.5

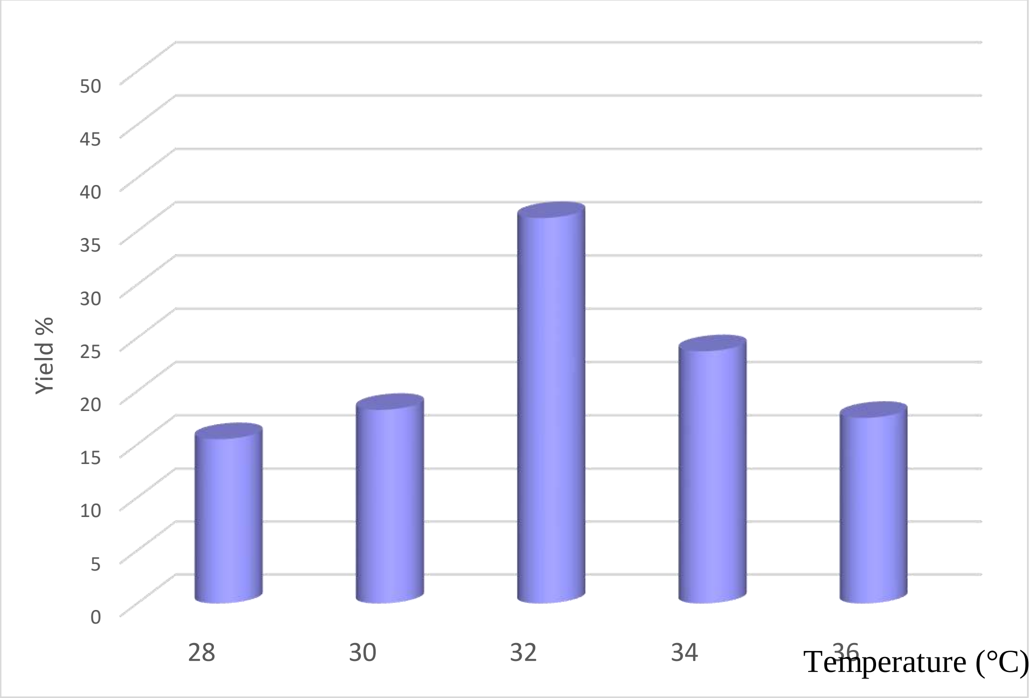

Figure III.10: Effect of temperature on bioethanol yield

III.4.4.3. Effect of the amount of yeast

The effect of the amount of yeast on bioethanol yield in different amounts was investigated (0.25, 2 g) by other fermentation conditions constant was used (pH 5, 32 °C).

The result is shown in figure (III.11). The amount of yeast increases from 0.25 %w/v to 1 %w/v bioethanol yield increase from 8.75 % to 36.25 % on the order after 48 hours of fermentation. Further increase in amount of yeast beyond 1 %w/v resulted in decrease of bioethanol yield. This may be as a resultof more yeast consuming the limited glucose for selfsustenance, there by resulting in low yield of bioethanol [14].

Table III. 8: Effect of the amount of yeast on bioethanol yield

The amount of yeast (g)	0.25	0.5	1.0	1.25	1.5	2.0
Bioethanol yield (%)	8.75	11.5	36.25	17.75	16.75	15.55

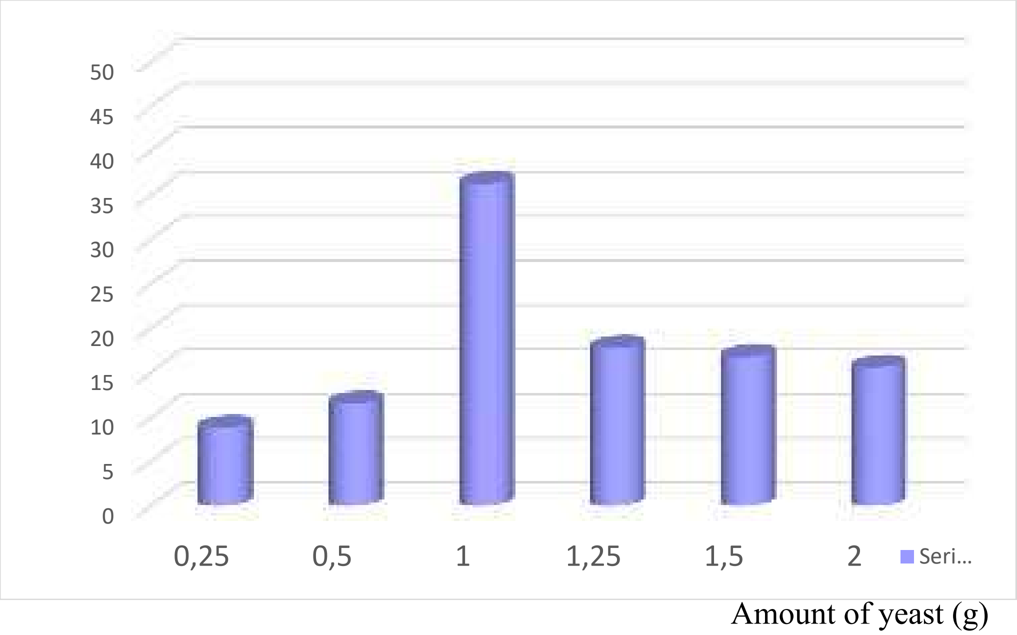

Figure III.11: Effect of the amount of yeast on bioethanol yield

III.4.4.4. Effect of time

This experiment was done to determine the time of which maximum bioethanol yield and the amount of yeast that gave the optimum bioethanol yield and was used for varying the fermentation duration of 1,2,3, days bioethanol at temperature of 32 °C, pH of 5.0. Figure (III.12) shows the effect of time on bioethanol yield. It can be seen from the figure (III.12) that give the best yield 36.25 % at (2 days) 48 hours of fermentation time.

Table III.9: Effect of time on bioethanol yield

Time (h)	24	48	72
Bioethanol yield (%)	18.75	36.25	21

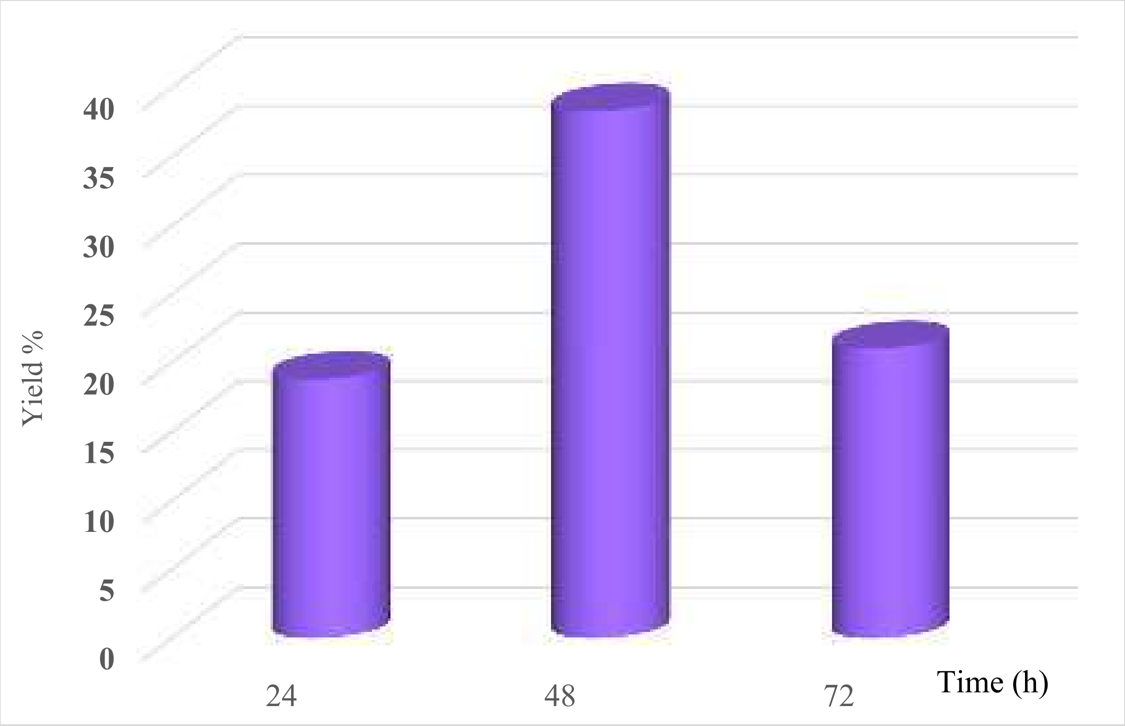

Figure III.12: Effect of time on bioethanol yield

III.4.4.5. Results of optimization of bioethanol

The results of the optimization of bioethanol production are tabulated in the table (III.10) that shows that the best optimum fermentation conditions were obtained at 32°C, pH 5.0, yeast amount 1g, and fermentation time of 48 hours (2 days), producing a bioethanol yield of 38.25%.

Table III.10: the optimization of bioethanol production

N°	t (h)	Q (g)	T(°C)	pH	Y(%)
1	24	1	32	4	18.75
2	24	1	32	4.5	25
3	24	1	32	5	36.25
5	24	1	32	5.5	21.25
6	24	1	32	6	17.5
7	24	1	28	5	15.5
8	24	1	30	5	18.25
9	24	1	32	5	36.25
10	24	1	34	5	23.75
11	24	1	36	5	17.5
12	24	0.25	32	5	8.75
13	24	0.5	32	5	11.5
14	24	1	32	5	36.25
15	24	1.25	32	5	17.75
16	24	1.5	32	5	16.75
17	24	2	32	5	15.55
18	24	1	32	5	18.75
19	48	1	32	5	38.75
20	72	1	32	5	21

T: Temperature, t: Reaction time, Y: Bioethanol yield, Q: Amount of yeast (g)

III.4.5. Comparison with previous studies

The bioethanol yields at optimum conditions by using different type date are tabulated in the table (III.11). The highest bioethanol yield (860.43 ml/Kg) by Hamraya while the lowest yield (136,6ml/Kg) was achieved by (Mech-Degla). As for the type of Degla bayda, we note from the table (III.11) that it is the third-best type of dates on the one hand of bioethanol production and in less time (48 h) compared to other types

Table III.11: Comparisons the bioethanol yield between our study and previous studies by different type the date

Feedstock	Yeast strain	Fermentation condition	Yield (ml/Kg	Ref.
Mech-Degla	*S. cerevisiae*	30°C, pH=4.5, 72h	136,6	[15]
Ghars	*S. cerevisiae*	32°C, pH=4, 72h	624	[5]
Tinicine	*S. cerevisiae*	32°C, pH=4, 72h	242	[5]
Taquermeste	*S. cerevisiae*	32°C, pH=4, 72h	333	[5]
Boucheire	*S. cerevisiae*	32°C, pH=4, 72h	475	[5]
Lebghel	*S. cerevisiae*	30°C, pH=4.7, 72h	250	[16]
Teggaza	*S. cerevisiae*	30°C, pH=4.7, 72h	235.7	[16]
Degla-Bayda	*S. cerevisiae*	30°C, pH=5, 48 h	365.7	This study

III.4.6. Results of product quality analysis

III.4.6.1. Results of FTIR analysis

An infrared spectrometer (IR) is an analysis technique used to verify the purity of bioethanol obtained (Fig. III.13 and table III.12).

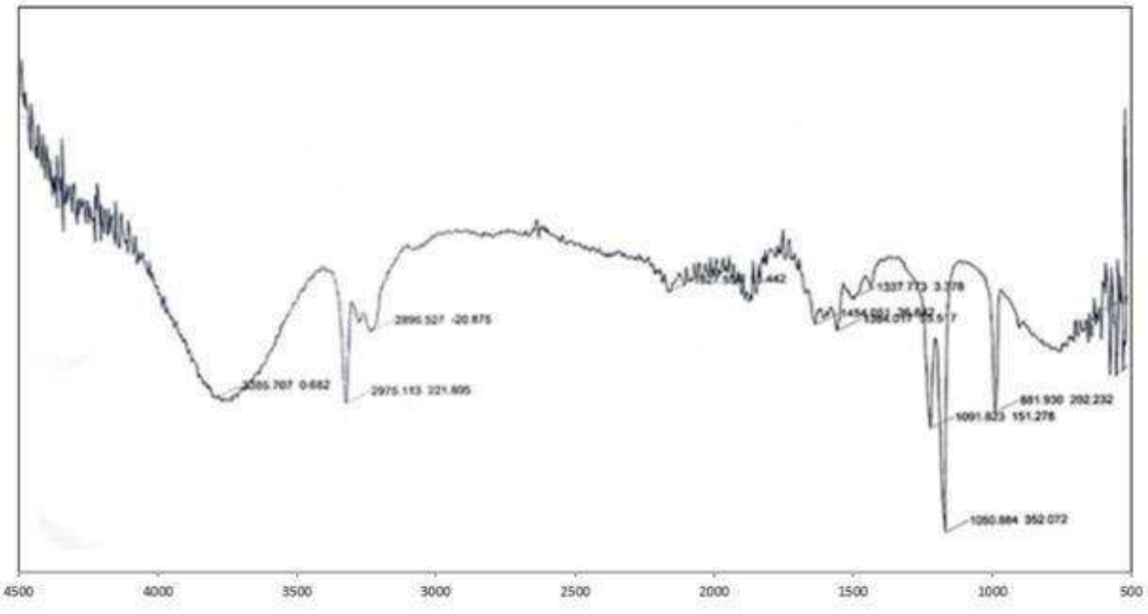

Figure III.13: IR spectroscopy of bioethanol produced

Figure (III.13) shows the spectral of bioethanol is identical in the spectral range between 4000 and400 cm-1.It is found that the spectrum shows a wide band at 3385.707 cm^{-1} which represents the elongation of the group OH [17]. They also have two bands medium and low in 2970,113 and 2895,527 cm^{-1} corresponding to the elongation of the CH_3 bond of CH_3 and CH_2, the elongation of the C-C bond (linear chain) that is confirmed by a mean peak at 881cm^{-1}. Finally, we observe that the intensities of the absorbance at the specific strong band at 1050 cm^{-1} corresponding to the elongation of the C-O bond of a primary alcohol [18]. The table (III.12) shows the results of the bioethanol base bands by IR, we notice similarities between the bands and the spectrum, indicating that our product conforms to international standards.

Table III.12 Most important absorption bands of the mid-infrared spectra of bioethanol

Wave number (cm^{-1})	Vibration
3385	O-H stretching of bonded and non-bonded hydroxyl grou
2975	Asymmetric C-H stretching
2896	Symmetric C-H stretching
1650	O-H bending
	Conjugated C-O stretching
1454	C-H deformation
1429	C-H deformation
	CH$_2$ scissoring
1384	Symmetric C-H deformation
1337	C-H vibration
	CH$_2$ wagging
1090	C-O stretching
1053	C-O stretching

III.4.6.2. Results of physicochemical properties

The production of bioethanol alone is not enough, it is necessary to ensure the quality of the product prepared by comparing it with international standards. In general, international standards used to know the quality of bioethanol are ASTM D 4806 and ASTM D 5798. We chose the properties according to the availability of the equipment in the laboratory [10]. The analysis of the results presented in table III.13 that shows that physical state, color and pH values are in line with European Standards. Density, boiling point of our bioethanol is close to the ethanol characteristics.

Table III.13: Characteristics of the bioethanol obtained

Characteristics	**Bioethanol**	**Ethanol**
Physical state	liquid	liquid
Color	Colorless	Colorless
pH	6.9	6.5-7.0
Density (g/cm^3)	0.82	0.79
Viscosity mPa.s	1.4	1.2
Boiling Point	78.5 °C	79 °C

III.5. Conclusion

In conclusion, the results obtained for this research showed that we can dates of “Degla Bayda” as a low-cost feedstock for bioethanol production and can be conclude that commercial *Saccharomyces cerevisiae* can use for bioethanol production and the best optimum fermentation conditions obtained at temperature 32°C, pH 5.0, yeast ratio 1g and fermentation time of 48 hours (2 days), bioethanol yield of 36.25% was obtained in accordance with this study, developing countries can produce clean bioenergy from the natural resources untapped they have in their daily life.

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General conclusion

Due to the nature of the massive depletion of fossil fuels, which has caused enormous environmental damage and pollution of the air, sea and soil, and contributed to global warming through greenhouse gas emissions of greenhouses. As a result, environmental standards aimed at limiting its emissions were established. currently, biofuels are considered as an essential alternative energy source to fossil fuels and which make it possible to respond to the increase in polluting emissions and to cope with the depletion of fossil energy resources on everything that the consumption of fuel to increase in the last ten years.

The aim of this study was to evaluate some waste and low value feedstocks or harmful matters to the environment into green "bio" fuels because it depleted it in nature, and so the recycling these worthless materials to biofuel production with in low-cost methods.

We conclude from the results of the study of biodiesel production the transesterification is the most widely used chemical process for the production of biodiesel, the results showed that low-cost and high-quality fuel created by waste cooking oil with ultrasonic method was the percentage yield of biodiesel was 99.7% and this high biodiesel yield concludes of the waste oil that using NaOH as a catalyst. The optimum conditions for maximum yield in this study with the microwave method are: 1 wt.% catalyst concentration, 1:09 oil to methanol molar ratio, 6 min reaction time at 70°C and 800 rpm. also showed the physico-chemical characteristics and functional and FTIR and MS spectroscopy the biodiesel produced is of good quality compared to international standards

The results showed that we can use dates of "Degla Bayda" as a low-cost feedstock for bioethanol production and can be conclude that commercial *Saccharomyces cerevisiae* can use for bioethanol production and the best optimum fermentation conditions obtained at temperature 32°C: pH 5.0, yeast ratio 1g and fermentation time of 48 hours (2 days), bioethanol yield of 38.25% was obtained.

In conclusion, developing countries (with weak economy) such as Algeria can adopt clean bioenergy production from valuing the biomass, they possess in their daily untapped life to preserve their environment and contribute to raising their economy.

Finally, future prospects for this study to boost environmental and economic competitiveness of the biofuels industry in Algeria will be provided. However, there is a higher capacity for the use of biofuels to replace conventional petroleum fuels in Algeria. A wide range of local feedstocks, processes, and applications for liquid biofuels can be found at varying degrees of progress for biofuel production. For biodiesel, some less expensive catalysts can be used, such as: CaO which is produced from eggshells, as for bio-ethanol, newer methods will be used in the primary treatment, such as: ultrasonic and microwave method and activating yeast to improve production. The transportation applications of liquid biofuel and the cost of producing biofuels under optimal conditions for optimum productivity will be studied using a program aspen Hysys.

Appendix

Table 1: The standards of ASTM D 975 for petroleum diesel fuel

Property specification	Units	Diesel ASTM D975	Limits
Flash point	°C	ASTM D975	60–80
Cloud point	°C	ASTM D975	–15 to –5
Pour Point	°C	ASTM D975	–35 to -15
Cetane number		ASTM D4737	46
Density at 15 °C	Kg/m^3	ASTM D1298	820-860
Kinematic viscosity at 40°C	mm^2/s	ASTM D445	2.0 to 4.5
Acid number	Mg KOH/g	D664	0.5 max
Cold filter plugging point	°C	EN 590	–8
Oxidation stability	mg/L	ASTM D2274	25 mg/L max
Carbon residue	% m/m	ASTM D4530	0.2 max
Copper corrosion		ASTM D130	Class 1 max
Distillation temperature	°C	ASTM D86	370 max
Lubricity (HFRR)	m	IP 450	0.460 mm (max)
Sulphated ash content	% mass	D874	0.002 max
Ash content	% mass	ASTM D482	100 maxi
Water and sediment	% mass	ASTM	0.05 max
Free glycerine	% mass	ASTM D 6584	0.02 max
Total glycerine	% mass	ASTM D6548	0.24
Phosphorus	% mass	ASTM D4951	0.001 max
Sulphur (S 500 grade)	ppm	ASTM D5453	500 max
Carbon	% wt	ASTM D975	87
Hydrogen	% wt	ASTM D975	13
Oxygen	% wt	ASTM PS121	11
BOCLE scuff	g	ASTM D975	2000-5000
Boiling point	°C	ASTM-D7398	100-615
Saponification value	mg KOH/g	ASTM D5558	-370 max

Table 2: The standards of ASTM D6751 for biodiesel fuels

Property specification	Units	ASTM D6751	Limits
Flash point	°C	ASTM D 93	130 min
Cloud point	°C	ASTM D2500	−3 to −12
Pour Point	°C	ASTM D97	−15 to −16
Cetane number		ASTM D613	47 min
Density at 15 °C	Kg/m^3	ASTM D 1298	880
Kinematic viscosity at 40 °C	mm^2/s	ASTM D445	1.9–6.0
Acid number	mg KOH/g	ASTM D664	0.5 max
Cold filter plugging point	°C	ASTM D6371	Maximum +5
Carbon residue	% m/m	ASTM D 4530	0.050 max
Copper corrosion		ASTM D 130	3 max
Distillation temperature	°C	ASTM D 1160	360
Lubricity (HFRR)	m	ASTM D6079	520 max
Sulphated ash content	% mass	ASTM D874	0.002 max
Ash content	% mass	–	–
Water and sediment	% mass	ASTM D 2709	0.005 vol % max
Free glycerine	% mass	ASTM D 6584	0.02 maximum
Total glycerine	% mass	ASTM D6548	0.24
Phosphorus	% mass	ASTM D4951	0.001 maximum
Sulphur (S 15 grade)	ppm	ASTM D5453	150 maximum
Sulphur (S 500 grade)	ppm	ASTM D5453	500 maximum
Carbon	% wt	ASTM PS121	77
Hydrogen	% wt	ASTM PS121	12
Oxygen	% wt	ASTM PS121	11
Bocle scuff	g	ASTM PS121	> 7000
Total contamination	mg/kg	ASTM D5452	24
Boiling point	°C	ASTM-D7398	100-615
Saponification value	mg KOH/g	ASTM D5558	370 maximum

Table 3: The standards of EN 14214 for biodiesel fuels

Property specification	Units	EN 14214	Limits
Flash point	°C	EN ISO 3679	101 minimum
Cloud point	°C	–	–
Pour Point	°C	–	–
Cetane number		EN ISO 5165	51 minimum
Density at 15 °C	Kg/m^3	EN ISO 3675/	860–900
Kinematic viscosity at 40 °C	mm^2/s	EN ISO 3104	3.5–5.0
Iodine number	g I_2/	EN 14111	–
Acid number	mg KOH/ g	EN 14104	0.5 v maximum
Cold filter plugging point	°C	EN 14214	–
Oxidation stability	mg/L	EN 14112	3 h minimum
Carbon residue	% m/m	EN ISO 10370	0.3 maximum
Copper corrosion		EN ISO 2160	Class 1
Sulphated ash content	% mass	EN ISO 3987	0.02 maximum
Water and sediment		EN ISO 12937	500 mg/kg
Monoglycerides	% mass	EN 14105	0.8 maximum
Diglycerides	% mass	EN 14105	0.2 maximum
Triglycerides	% mass	EN 14106	0.2 maximum
Free glycerine	% mass	EN 1405/	0.02 maximum
Total glycerine	% mass	EN 14105	0.25
Phosphorus	% mass	EN 14107	0.001
Total contamination	mg/kg	EN 12662	24

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RESEARCH ARTICLE

Emerging techniques in Agricultural Waste Valorization in Bioethanol production

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ABSTRACT:

Bioethanol is the only liquid fuel for gasoline engines that is renewable and immediately available since it is produced from vegetable raw material, also called biomass. Hence the name bioethanol refers originally to living plant, not to fossil fuel. It is produced by the transformation of biomass sugars by yeast which is responsible for the fermentation of production alcohol. The objective sought through this study is valuation of biomass that is possibly made for any product with low market value obtaining bioethanol which constitutes a product of high added value, for example the bio valuation of dates of poor quality called " Degla Bayda ". Physicochemical analyzes were carried out from the dates mill and during its alcoholic fermentation. Identification and purity verification analyze were undertaken for bioethanol. The results obtained show that fermentation conditions obtained that gave the optimum bioethanol yield was chosen of 36.25% at temperature 32°C, pH 5.0, yeast ratio 1g and fermentation time of 48 hours and the quality of our product is conformed with international standards.

KEYWORDS: Valuation, poor-quality dates, fermentation, yeast, bioethanol.

INTRODUCTION:

Global energy demand and consumption are continuously increasing with the rapidly growing world population. This has resulted in a grave depletion of fossil-fuel reservoirs and The exploitation of fossil fuels is responsible for 73% of global carbon dioxide emissions which are considered to be the main factor contributing to global warming and environmental change[1,2]. Therefore, there has been an emergent need for alternative renewable fuel sources to replace fossil fuel based conventional energy sources[3].

These biomasses are a renewable, abundantly available, and low-cost feedstock on the renewable energy technology platform providing a great potential for production biofuels of second-generation[1]. Bio-fuels are attracting growing interest around the world, with some governments announcing commitments to bio-fuel programs as a way to both reduce greenhouse gas emissions and dependence on petroleum-based fuels. Bioethanol is by far the most widely used bio-fuel for transportation worldwide, because it is a renew- able, biodegradable resource, nontoxic, and it is oxygenated, thereby provides the potential to reduce particulate emissions in engines[4,5]. Among them, Bioethanol is nowadays increasingly used as ecofriendly is one of the most important biofuels generated from renewable resources such as corn mostly in the USA and sugarcane in Brazil as well as carob[6] . Bioethanol is ethanol extracted by fermentation, fermentation is anaerobic bioconversion in the presence of yeast and in appropriate

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conditions of temperature and pH, the fermentation of sugars contained in the biomass[7]. vegetable biomass as feedstock for bioethanol production allows for recycling the CO2 released during combustion, reducing the CO2 emissions[5]. Nowadays, the commercial bioethanol is almost entirely of first generation since food crops are used as feedstock: sugarcane in Brazil, corn in the US, and wheat and sugar beet in the European Union (EU), rice straw[8], tea processing waste[9,10], rice hull[11,12] , barley husk, wheat bran, rye bran[13], Ulva lactuca[14], and taro waste[15], and the production of ethanol is increasing every day[16]. The main disadvantage of first generation bioethanol is the competition over the utilization of arable land for cultivation of food crops between biofuel feedstocks, thus resulting on the increase of food prices[5,17]. In the present study is valorization of biomass is made possible for any product with low market value obtaining bioethanol which constitutes a product of high added value, and that from the bio-valuation of poor-quality dates called we chose " Degla Bayda " since is a poor quality in Algeria.

MATERIALS AND METHODS:

Collection of samples:

Dates (Degla Bayda) was collected from Mr. Babziz farm in the region of Ouargla in Algeria which is characterized by the production of a large quantity of dates. The collected dates were manually cleaned were cleaned by tap water and pitted. Afterward, the samples later and were cut into small pieces and served as a working sample for the experiment. A yeast commercial Saccharomyces cerevisiae were used for fermentation.

Extraction of date juice:

The juice is a sweet liquid that is prepared, the date is obtained by maceration of dates pitted in hot water for 5 hours. The quantity is determined by 400 g of date on each 1L of distilled water. Finally, the solution is filtered to separate the musts and dates, we get 400 ml of date juice[18].

Bioethanol fermentation:

Ethanol fermentation was carried out using 100 ml of date juice which the best hydrolysis treatment based on the number of sugars produced from date. Yeast is used *S. cerevisiae* in the fermentation process by different the amount at under various conditions of pH and temperature and time for the best yield of bioethanol production[18,19].

Distillation and rectification:

At the end of the fermentation, a date wine is obtained which must be filtered to separate the yeast. Fractional distillation is a process used to separate ethanol from water according to their different volatilities. This process essentially consists of boiling the ethanol-water mixture. It is distilled at a temperature of about 80 °C and 78 ° C[3,7].

Bioethanol Yield:

We calculated the yield of bioethanol extracted using the following relation:

R=(Quantity of Alcohol (v))/(Quantity of Date (w))×100

Bioethanol characteristics:

In order to see if our extracted bioethanol complies with international standards, we have analyses physic-chemical parameters for our bioethanol, the parameters we used are: Physical state, color, pH, density, Boiling Point[20,21].

IR spectrometry:

The FT-IR spectra were recorded using Agilent Resolution Pro spectrometer at ambient temperature in the wave number 400-4000 cm^{-1}.

RESULTS AND DISCUSSION:

Bioethanol Yield:

The effects of pH, temperature, time, and ratio of yeast on the ethanol yield, substrate consumption and product formation rates were analyzed in fermentation[22]. Figure 1,2,3, and 4 show the experimental results for the fermentation.

Effect of pH on Bioethanol Yield:

The effect of pH on ethanol fermentation was studied at different pH ranging from pH 4.0 to 6.0 for used yeast strain namely commercial *S. cerevisiae*, incubation was carried out at 32°C, for 24 hours of fermentation period. Glucose conversion was continuing simultaneously during the fermentation. As shown in (Fig.1) the yield of ethanol was increased between pH 4.0 to 5.0 and then decreased below this value. The maximum of yield ethanol 36.25 % at pH 5.0.

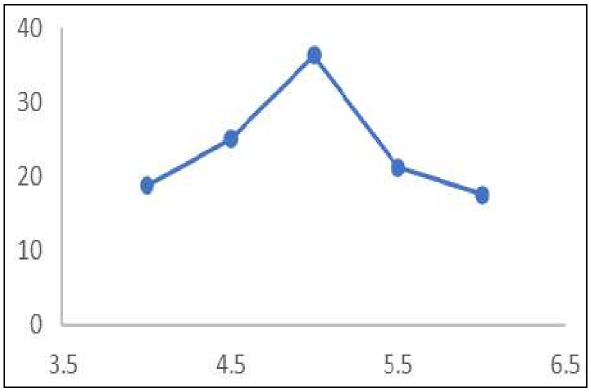

Figure.1: Effect of pH on Bioethanol Yield

Effect of Temperature on Bioethanol Yield:

Bioethanol production affected by the temperature variation. Under suggested fermentation protocol, commercial S. cerevisiae Yeast strain showed diverse results under different temperature conditions (28℃, 30℃, 32℃, 34℃, 36°C) with regarding to bioethanol production (Fig.2). The best bioethanol yield was reported 36.25 % at 32 ℃, while at 30℃ and 34°C, bioethanol productions were 18.25% and 23.75 % On the order, a reduction in ethanol production was occurred at 28°C and 36°C was by 15.5%, 17.5% On the order. These results may be due to the reduction in the growth of S. cerevisiae yeast at higher and very low temperature that too decreased the rate of fermentation process.

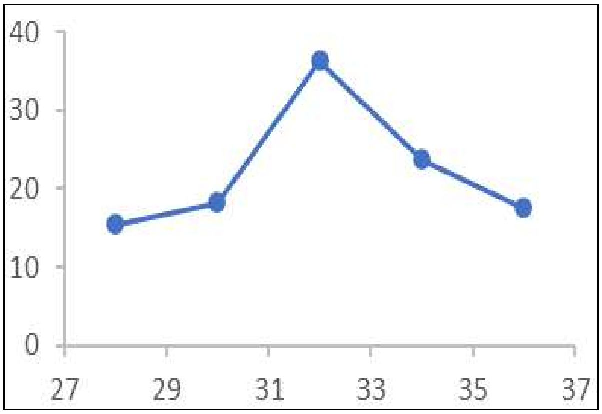

Figure.2: Effect of Temperature on Bioethanol Yield

Effect of the amount of yeast on bioethanol yield:

The effect of the amount of yeast on bioethanol yield in different amounts was investigated 0.25, 0.50, 1.0, 1.25, 1.5, 2.0g by other fermentation conditions constant was used (pH5, 32°C). The result is shown in Figure 3, which showed that as amount of yeast increases from 0.25%w/v to 1%w/v bioethanol yield increase from 8.75% to 36.25% on the order after 48 hours of fermentation. Further increase in amount of yeast beyond 1%w/v resulted in decrease of bioethanol yield. This may be as a result of more yeast consuming the limited glucose for self-sustenance, thereby resulting in low yield of bioethanol.

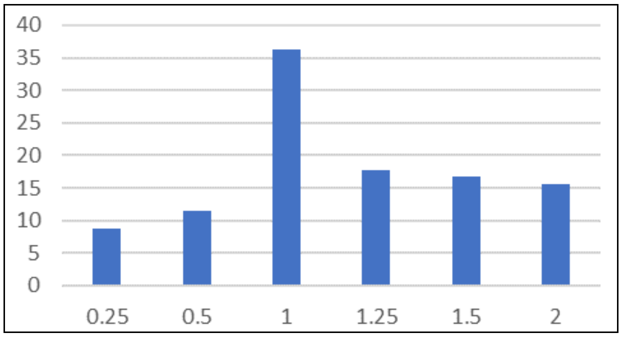

Figure. 3: Effect of the amount of yeast on bioethanol yield

Effect of time on bioethanol yield:

This experiment was done to determine the time of which maximum bioethanol yield and the amount of yeast that gave the optimum bioethanol yield and was used for varying the fermentation duration of 1, 2, 3, days bioethanol at temperature of 32°C, pH of 5.0. Figure 4 shows the effect of time on bioethanol yield. It can be seen from the figure that give the best yield 36, 25% at (2 days) 48 hours of fermentation time.

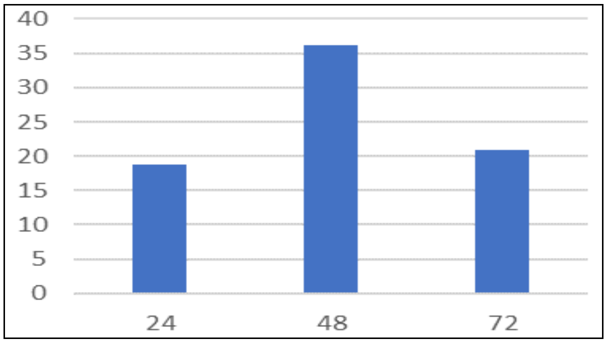

Figure. 4: Effect of time on bioethanol yield

Bioethanol characteristics:

The production of bioethanol alone is not enough, it is necessary to ensure the quality of the product prepared by comparing it with international standards. In general, international standards used to know the quality of bioethanol are ASTM D 4806 and ASTM D 5798. We chose the properties according to the availability of the equipment in the laboratory[21].

Table.1: The characteristics of the bioethanol product obtained

Characteristics	Bioéthanol	Ethanol
Physical state	Liquid	Liquid
Color	Colorless	Colorless
pH	6.9	6.5-7.0
Density (g/cm^3)	0.82	0.79
Boiling Point	78.5 °C	79 °C

The analysis of the results presented in Table 2 shows that Physical State, Color and pH values are in line with European standards. density, boiling point of our bioethanol is close to the ethanol characteristics.

FT-IR spectrometry:

An infrared spectrometer (IR) is an analysis technique used to verify the purity of bioethanol obtained (Fig.5 and Table.2)

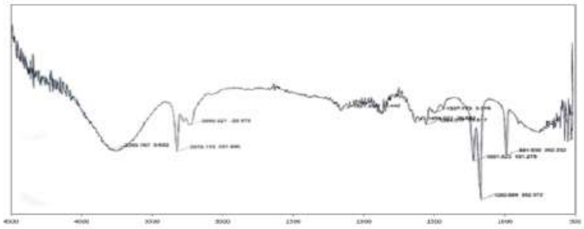

Figure.5: IR spectroscopy of bioethanol produced

Figure 4 explain the spectral of bioethanol is identical in the spectral range between 4000 and 400 cm^{-1}.It is found that the spectrum shows a wide band at 3385.707 cm^{-1} which represents the elongation of the group O-H[23].They also have two bands medium and low in 2970,113 and 2895, 527 cm^{-1} corresponding to the elongation of the CH_3 bond of CH_3 and CH_2, the elongation of the C-C bond (linear chain) that is confirmed by a mean peak at 881cm^{-1}. Finally, we observe that the intensities of the absorbance at the specific strong band at 1050 cm^{-1} corresponding to the elongation of the C-O bond of a primary alcohol[24]. The table 2 shows the results of the bioethanol basebands by IR, we notice similarities between the bands and the spectrum, indicating that our product conforms to international standards.

Table 2 Most important absorption bands of the mid-infrared spectra of bioethanol

Wavenumber (cm^{-1})	Vibration
3385	O-H stretching of bonded and non-bonded hydroxyl groups
2975	Asymmetric C-H stretching
2896	Symmetric C-H stretching
1650	O-H bending
	Conjugated C-O stretching
1454	C-H deformation
1429	C-H deformation
	CH_2 scissoring
1384	Symmetric C-H deformation
1337	C-H vibration
	CH_2 wagging
1090	C-O stretching
1053	C-O stretching

CONCLUSION:

In conclusion, from the results obtained for this research showed that we can dates of “Degla Bayda” as a lowcost feedstock for bioethanol production and can be conclude that commercial *S. cerevisiae* can use for bioethanol production and the best optimum fermentation conditions obtained at temperature 32°C, pH 5.0, yeast ratio 1g and fermentation time of 48 hours (2 days), bioethanol yield of 36.25% was obtained.in accordance with this study, developing countries can produce clean bioenergy from the natural resources Untapped they have in their daily life.

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