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Real-time monitoring of the transesterification reaction in biodiesel production

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Abstract

The massive industrialization era led to an uncontrolled consumption of the world resources and brought the present world to a necessity: find alternative fuels in order to reduce the environmental changes. Two important biofuels are present in the market at the moment: bioethanol and biodiesel. Because of their high price, they are not used pure but only poorly mixed in blends.
In this thesis it is proposed an indirect way to monitor the reaction through the on-line analysis of the viscosity instead of the manual sampling from the reactor. The physical property analysis, instead of the chemical one will result in a cheaper, faster way to determine the reaction's end point, improving the safety inside the plant and increasing its productivity.
It is hope that this study will help do reduce the price of biodiesel. This will result in greener affordable blends, richer in biofuels.
In this work the biodiesel production, using transesterification reaction from rapeseed oil, is described and studied. It is then analysed the relation between the viscosity behaviour inside the reactor, while the reaction take place, and the formed products. The most important characteristic of this monitoring method is that, using some calibrations curves opportunely made for that particular plant, it is possible to use it on-line. This characteristic avoids the necessity of continuous sampling of the mixture through time and can automatically provide to the plant operators the conversion of the reagents and the quantity of the products through the funded relations. Furthermore, it has been studied, changing the reaction conditions, that this relation can be found in different conditions and environments.
This study was performed with a cylindric viscometer placed inside a 250 ml reactor. The viscometer was used to detect the viscosity inside the reactor at different times while the same mixture was sampled, quenched and then analysed with HPLC. The HPLC chromatograms were than compared with the viscosity value in order to find the searched relation. The results confirm the previous assumptions and detected a relation between the variables. The resulted values suggested that it was possible to fit some parameters and find the final relation between the viscosity and the FAMEs area.

The experiments in this thesis were performed during an ERASMUS+ period at the University of Coimbra supervised by Prof. Abel Ferreira and Prof. Marco Seabra Dos Reis.
All the feedstock used was provided by the Portuguese company PRIO, together with the biodiesel reference samples.

Riassunto

Il consumo mondiale incontrollato delle materie fossili per la produzione di energia ed il trasporto, esploso negli ultimi decenni, ha portato il mondo in una situazione critica. Da anni si cercano quindi fonti alternative di combustibili per poter ridurre la dipendenza mondiale da quelli tradizionali. I Biocombustibili rappresentano un'ottima alternativa a quelli fossili, essendo essi provenienti da biomasse e quindi rinnovabili. Due biocombustibili, biodiesel e bioetanolo, sono già presenti nel mercato globale in grandi quantità. Il loro costo però non permette il loro utilizzo da composti puri ma soltanto mescolati all'interno di blend, assieme ai più economici combustibili tradizionali. Il tentativo ultimo di questo lavoro è la riduzione, anche minima, del prezzo del biodiesel. Ogni piccola riduzione del prezzo porterà ad una maggiore possibilità di industrializzazione di quest'ultimo risultando quindi in blend più ricchi in biodiesel pur mantenendo il prezzo accessibile al consumatore finale.
In questo lavoro è stata descritta e studiata la produzione del biodiesel attraverso la reazione di transesterificazione. In particolare, è studiato un metodo promettente in grado di monitorare la produzione del biodiesel attraverso il cambiamento della viscosità durante il tempo di reazione. Essendo solitamente utilizzato un reattore batch per la produzione del biodiesel, è importante determinare l'esatto momento in cui i reagenti, olio e alcool in questo caso, vengono consumati per formare i prodotti (Acidi grassi di esteri metilici). Viene quindi analizzato il comportamento della viscosità all'interno del reattore durante l'avanzamento della reazione. La relazione tra viscosità e la formazione dei prodotti può infatti essere determinata durante il tempo. Questo dà la possibilità, analizzando solo la viscosità all'interno del reattore, di determinare l'esatto momento in cui i reagenti sono consumati e si formano i prodotti. La parte più importante di questo sistema di monitoraggio è che, usando una curva di calibrazione specifica per l'ambiente di reazione studiato, esso può essere utilizzato direttamente online, ossia dentro al reattore. Questa precisa caratteristica andrebbe a evitare la necessità di campionare continuamente la miscela interna al reattore, fornendo all'operatore i valori di conversione e la quantità dei prodotti già formati senza l'uso di analisi complesse e in maniera più sicura. Inoltre, si è studiato che questa relazione non è univoca per il tipo di olio o ambiente di reazione preso in considerazione, ma può essere analizzata in varie condizioni e in diversi ambienti di reazione.
In questo caso le reazioni sono state eseguite all'interno di un reattore da 250ml nel quale era inserito un viscosimetro cilindrico. Il viscosimetro misura la viscosità della miscela in determinati valori di tempo mentre, contemporaneamente, viene estratto un campione che poi è analizzato in HPLC. I cromatogrammi dei vari campioni sono poi confrontati con i valori di viscosità così da trovare la relazione cercata.
I valori risultanti suggeriscono l'esistenza di alcuni parametri che determinano la relazione tra la viscosità del fluido e la produzione di biodiesel, confermando quindi le ipotesi iniziali.

Questo lavoro è stato svolto durante un periodo di studio ERASMUS+ presso l'Università di Coimbra sotto la supervisione di Prof. Abel Ferreira e Prof. Marco Seabra Dos Reis.
L'olio di colza necessario per gli esperimenti e il biodiesel di riferimento è stato fornito dalla compagnia portoghese PRIO.

Summary

Introduction

Considering the current energy consumption scenario (IEO2018 Reference Case), in which the increase of demand of fossil fuel is much higher than the discovery of new sources, alternative energy began to appear as a way to supply the demand.

Considering also the environmental problems that our society it is facing, since the massive industrialization era, the world requires alternative fuels that are less harmful on global warming and climate changes.

For this reasons, greener fuel such bioethanol and biodiesel have been extensively studied. Biodiesel in particular, is considered a renewable, clean and biodegradable fuel that contains less pollutants compound than conventional fuels.

Biodiesel and bioethanol are already used in the European market blended with the more conventional petrodiesel.

However, the cost of production of these greener fuels is one of the main reasons of why they are not yet applied like a complete substitute of the conventional fuels.

For these reasons the reduction of the biodiesel's price of production is important.

One of the costs that every industry producing biodiesel must face is the monitoring of the conversion of the triglycerides during the process and the quality of the products. The analysis is commonly done by chromatographic method: this kind of measurement is suitable for laboratory scale analysis, provided detailed composition of the product. However, several drawbacks are present for this type of analysis: high costs, extensive sample preparation and lack real-time indications.

In order to keep monitored the production of fatty acids methyl esters (FAME), the main products inside the reaction, alternative methods of quality analysis have been studied. Among all of them, a notable correlation has been found relating the quantity of methyl ester formed and the viscosity of the mixture inside the reactor.

This correlation can be utilized for the online monitoring of the reaction, and in particular an easy and cheap way to monitor various types of transesterification reaction performed with different feedstocks and inside different environments.

The main idea of this work is that, analysing the time variation and following the trend of the viscosity directly inside the reactor, it will be possible to determine the conversion of triglycerides and the relative production of fatty acid methyl esters (FAMEs).

Chapter 1

Biodiesel production

A large variety of Biofuels have been studied in order to substitute the conventional fuels. One of them gained a lot of market in the last decade: biodiesel. However, despite all the environmental benefits that could derive from its use, biodiesel cannot be extensively applied as a complete substitute fuel for conventional diesel engine. One of the main reasons is the cost of its production.

1.1 The rise of fuels consumption

Due to the growing of the global population, the energy request is increasing year after year, and, even if the research of alternative ways to provide energy for the planet have made huge steps forward, the dominant resources for world energy supply still are crude oil, coal and natural gas.

In Figure 1.1 it is possible to analyse the historical evaluation and predicted increasing of the energy consumption divided between OECD and non-OECD nations. The OECD nations are trying to reduce the growth of the consumption, but the effort is not shared by the others non-OECD countries.

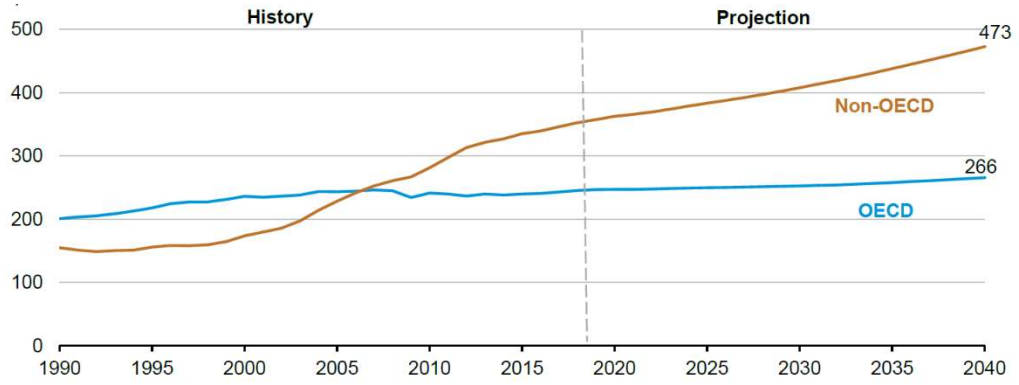


Figure 1.1.: *World energy consumption in quadrillion Btu. (Source: IEO2018 Reference case)*

Taking the U.S for example, Figure 1.2 (b) shows which kind of fuels are being used by this country in order to provide the energy needed by all the sectors. Since energy consumption is expected to increase in the coming years, the graphic shows that the consumption of the various fuels will not decrease, it will increase or at least it will remain stable. The only exception seems to be the case of Coal.

Focusing on the consumption of liquid biofuels, such as bioethanol and biodiesel, after the initial gain of a market share in the early 2000, is expected to remain stable in the next decades.

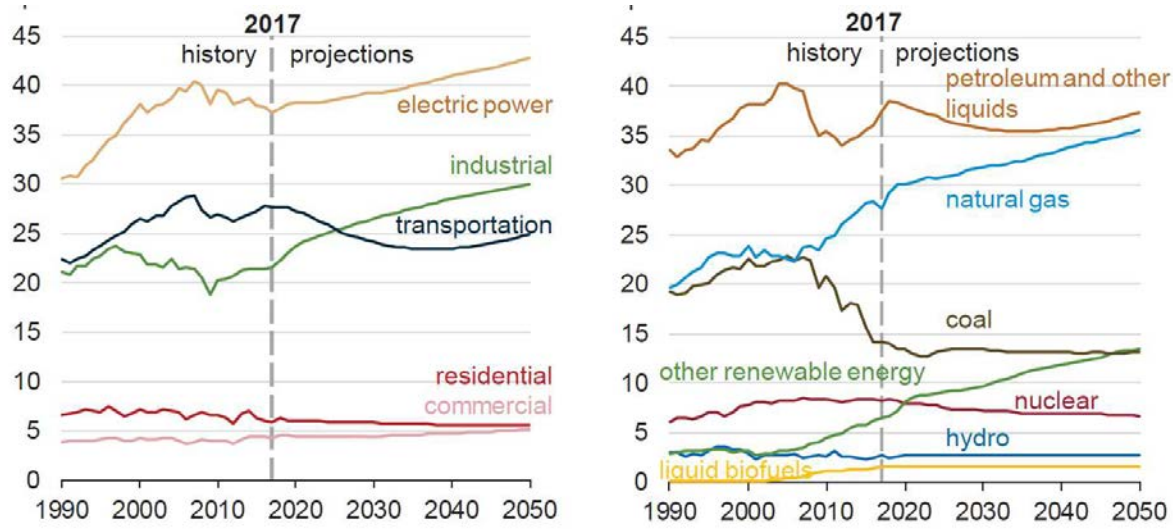


Figure 1.2. *(a) U.S. Energy consumption by sector; (b) Energy consumption by fuel; in quadrillion Btu (Source: IEO2018 Reference Case)*

The consumption of energy can arise from many fields. Figure 1.2. (a) shows how the energy is divided between the different area of consumption. Interesting is the transportation sector behaviour: even if it's not growing like it was in the past years, it still represents a big share of the energy consumption.

Although the share of total energy used for transportation declines over the projected period, from 96% in 2012 to 88% in 2040, petroleum and other liquid fuels are expected to remain the dominant sources.

Before 2040 it's expected that the transportation sector consumption of liquid fuel, in absolute terms, will show a growth of $36 \times 10^{12}\ MJ$, with diesel (including biodiesel) showing the largest gain ($13 \times 10^{12}\ MJ$), jet fuel consumption increasing by $10 \times 10^{12}\ MJ$ and motor gasoline (Including ethanol blends) increasing by $9 \times 10^{12}\ MJ$.

As shown in Figure 1.3., the diesel fuels are expected to remain the second largest transportation fuel for the years to come (EIA, 2016).

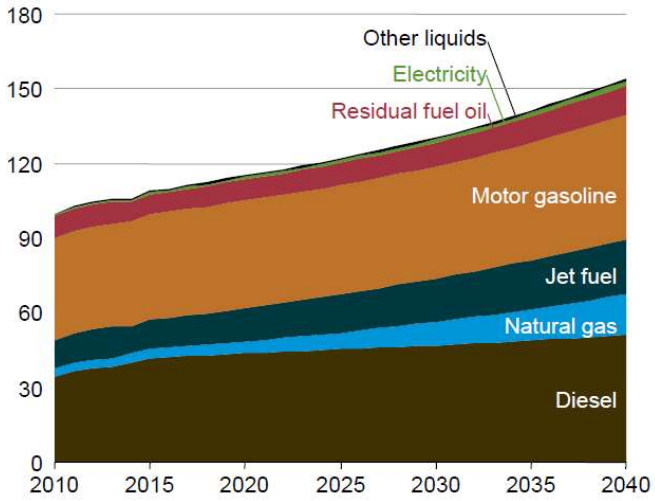


Figure 1.3. *World transportation sector delivered energy consumption by energy source, 2010–40 (quadrillion Btu) (Source: IEO2016 Reference Case)*

Knowing that diesel will be the second most important fuel in the next years, new kind of fuels has been studied to provide greenest alternatives to the common oil based diesel. These alternative fuels are commonly known as *biofuels*.

1.2 Biofuels

Biofuels refers to solid, liquid and gas fuels produced through fermentation, gasification, pyrolysis, torrefaction or by chemical reaction of a biological feedstock.
Biofuels can be divided in three categories:

- *First generation biofuels:* produced from oils, sugars and starches; this type of biomass compete with humans and animals' alimentation, so there are a lot of controversies regarding the use of these sources;
- *Second generations biofuels:* produced form non-food crops such as perennial grasses and woody materials, and the non-food portion of food crops. With this type of sources, the problems do not regard the human alimentation but the destruction of the world biodiversity and the quantity of residues that the cultivation of this biomass can cause are handicaps;
- *Third generations biofuels:* produced from special energy crop such algae. Algae's production seems to be very promising, acting like a high energy source and an entirely renewable feedstock. The problems related to this type of biomass seem to be the economic one. In fact the scale-up of algal system for biofuel production is very expensive (Nelson and Starcher, 2017);(Bhargavi, Nageswara Rao and Renganathan, 2018) .

Knowing that it is not possible to rely on fossil fuel forever, in the last 20 years this kind of alternative fuels, the Bio one, start gaining an interesting market share: primarily bioethanol in the case of the spark-ignition engine and vegetable oil, like biodiesel, in the case of compression ignition engine.

Bioethanol is a liquid biofuel produced from the fermentation and distillation of feedstock derived from annual food crops. Annual row crops are grown and harvested in a single year and must be planted every year. Sugarcane is the primary feedstock in Brazil, and corn grain is the primary feedstock in the United States. These two feedstock sources are converted into approximately 62% of the world's bioethanol.

In the *Biodiesel* case, the primary agricultural sources of lipids are annual row crops soybean, palm, and oilseed rape. Soybean is the primary feedstock source for first-generation biodiesel produced in the United-States, Europe, Brazil and Argentina, which are world leader in biodiesel production. The biodiesel production process will be described in the next chapter.

With the years also the Biogas, the gas produced by the fermentation of the organic materials by anaerobic microorganism that can be used to produce electricity, heat and as transportation fuel, start gaining a little share of the total biofuel market (Scarlat, Dallemand and Fahl, 2018).

The consumption of the different kinds of biofuel is different between countries. For example, while in the US the major biofuel produced and used is the *methanol,* in the EU, the most commonly used is the *biodiesel*.

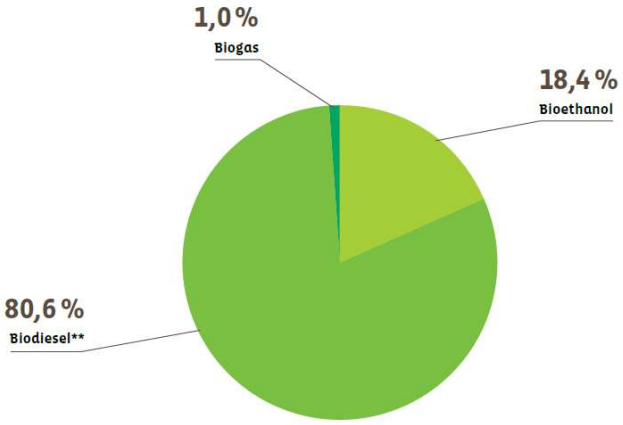


Figure 1.4. *Breakdown of total EU 2016* biofuel consumption in energetic content for transport by biofuel type. (Source Barometer,2016)*

The Figure 1.4. shows the division of the 2016 biofuels total consumption (14.4Mtoe) inside the European union: 80.6% (79.8%in 2015) of biodiesel, 18.4% (19.2% in 2015) of bioethanol and 1% of biogas (Barometer, 2016).

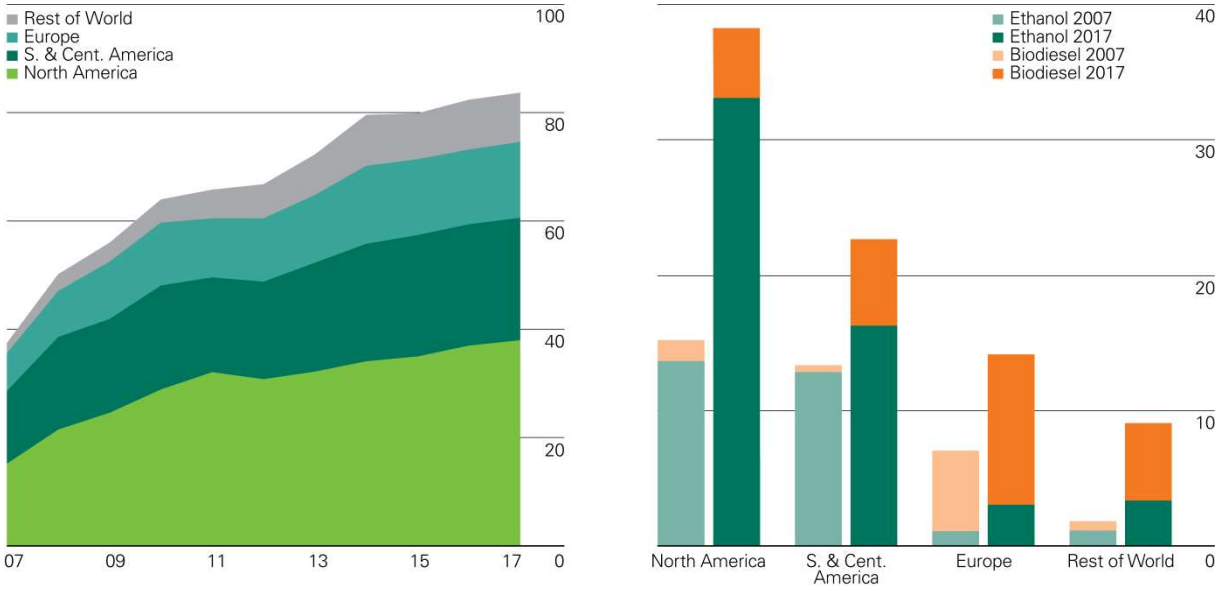


Figure 1.5. *Growth of biofuels production from 2007 to 2017 (Source: BP Statistical review of world energy June 2018)*

As shown in Figure 1.5. focusing on the past 10 years the production of biofuel has increased in almost all market.

Focusing on biodiesel, the region with the biggest market share in 2017 was Europe which, also nowadays, confirms itself as the major producer worldwide.

1.3 Biodiesel

Biodiesel is a biofuel for use in direct injection compression ignition engines. Although, the transportation industry is the one that uses most of the production of biodiesel, it can be used also as heating fuel, power generation, lubricants, plasticizers, high boiling absorbent for cleaning of gaseous industrial emission, as well as various solvent applications.
Part of the success in the introduction of biodiesel into the market has been due to the great variety of feedstocks that can be used for its manufacture (Knothe and Razon, 2017).

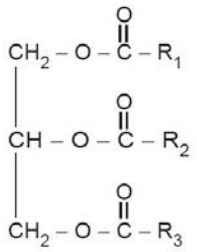


Figure 1.6. *Chemical structure of a triglyceride (Schumacher, 2004).*

Biodiesel refer to the methyl and ethyl alkyl ester (FAME, FAEE) produced from a transesterification of a long chain fatty acid and alcohol. The sources of the fatty acids are the triglycerides present in vegetable oil, animal fat, non-edible plant oil and waste cooking oil. In Figure 1.6. a typical chemical structure of a triglyceride is shown, where the R1, R2 and R3 are hydrocarbon chains that changes within the type of feedstock.
Also, even if it's not treated in this work, is right to say that a new biodiesel sector came onto the scene recently. In this case the fuel is derived using a different conversion process, from hydrogenated vegetable oils (HVO).
Biodiesel can be blended with petroleum diesel to create a biodiesel fuel.

1.3.1 Properties of Biodiesel

Biodiesel's chemical properties are define by: fatty acid content, aromatics, olefins, paraffins, carbon, hydrogen, oxygen and sulfur content, acid neutralization number, iodine number, and Conradson carbon residue number (Schumacher, 2004).
These properties usually change within the type of feedstock that is used. Knowing the chemical structure of the triglyceride it is possible to speculate the property of the final product.
Based on the studies about biodiesel made in the past years, it's possible to say that it is essentially free of sulfur and aromatics. This lack of pollutants inside the biodiesel is an advantage because they are responsible for the poisoning of the catalytic converter technology that is used to reduce engine exhaust emission and they are compounds that are believed to be carcinogenic (Schumacher, 2004).

In biodiesel paraffins and olefins are also present. These compounds are responsible of the increasing of the cold flow of biodiesel and the fuel oxidation respectively. Luckily the presence of this kind of compounds results lower than the normal petroleum diesel.
Biodiesel is also characterized by high flash point, inherent lubricity, biodegradability, miscibility with petroleum diesel in all blend ratios and compatibility with the existing fuel distribution infrastructures.
As shown in Table 1.1 the use of biodiesel tends to reduce the amount of harmful emissions when compared to the normal fuels. In this table the difference of emissions between biodiesel and petrodiesel of three different common engines are shown.

Table 1.1. *Changes in Regulated Emission with Biodiesel (Sharp, Howell and Jobe, 2000)*

Engine	HC	CO	PM	NO_x
Cummins N-14	-95.6	-45.3	-28.3	+13.1
DDC S-50	-83.3	-38.3	-49.0	+11.3
Cummins B5.9	-74.2	-38.0	-36.7	+4.3

While the biodegradability of biodiesel is always considered as an advantage in case of spilling in the environment, this also means that bacterial biomass may also grow during storage and form deposit that could clog engines and lines.
The overall environmental benefit or damage of the production and usage of biodiesel must be evaluated by a life cycle analysis (LCA). Biodiesel from terrestrials' sources is generally computed to have a positive energy balance: more fuel energy is derived from the product than was consumed to produce it. For example, a life-cycle analysis for soybean-derived biodiesel showed positive energy ratio and a review of palm biodiesel LCA showed uniformly positive energy ratios with dependence on system boundaries. On the other hand, aquatic feed-stock like microalgae may result in an almost negative energy balance (Knothe and Razon, 2017).
Furthermore, although some fossil-base CO_2 is released during the biodiesel production, mainly from the methanol consumed, the net production of CO_2 is reduced by 78% if compared with the fossil fuels one.

The technical challenges that are not yet resolved within the biodiesel are:

- The reduction of NO_x exhaust emissions;
- The improve of oxidative stability;
- The cold flow properties.

1.3.2 Transesterification

The transesterification is a chemical reaction where an alkoxy group of an ester is exchange with the one of an alcohol to form the new ester product. In the case of biodiesel, the

transesterification consists in the production of mono-alkyl esters from a vegetable or plant oil which is largely composed by triacylglycerols.

The reactant that, with the triacylglycerols concur to the formation of biodiesel can variate. In Figure 1.7. are shown the possible reactions that can bring to the formation of the FAMEs or FAEE, depending of the alcohol used. The latter is also responsible for the formation of the by-product that derive from the reaction, although the most common is glycerol.

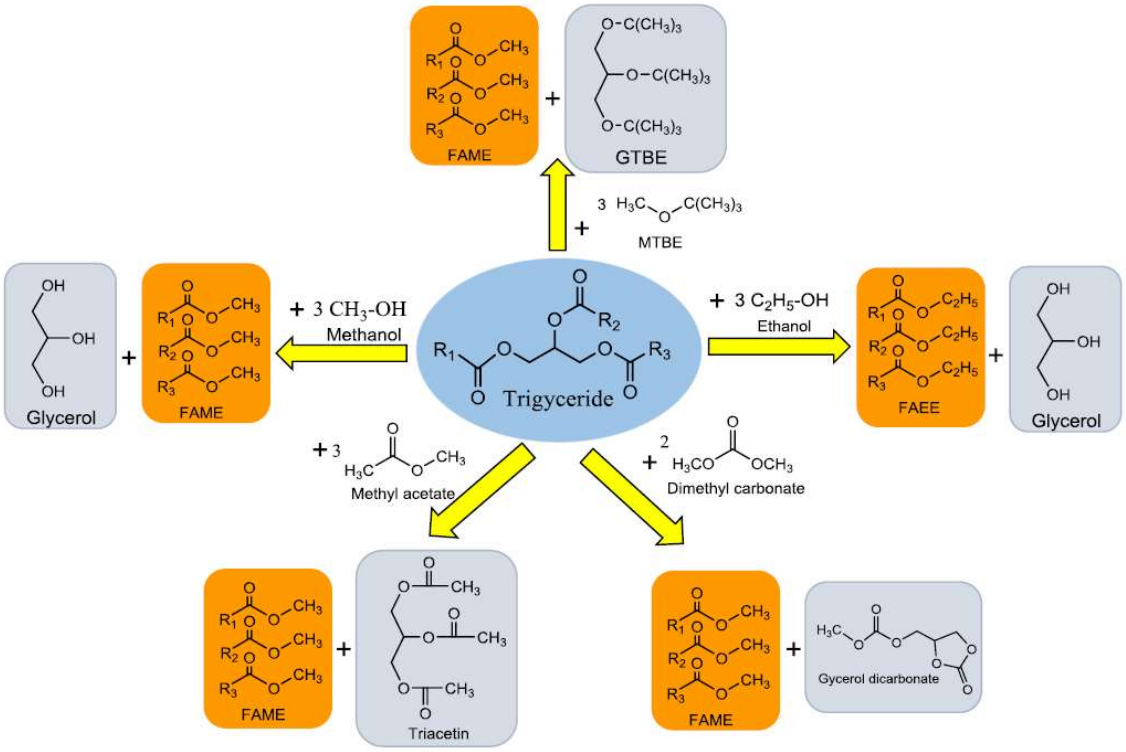


Figure 1.7. *Reaction of biodiesel production under different reactants (Farobie and Matsumura, 2017)*

All the reactants shown above can be used for the production of biodiesel but, while higher alcohols provide superior cold flow properties, they are generally more difficult to produce, require higher temperatures, lower levels of water contamination, and more complex alcohol recycling due to the formation of azeotropes (Schumacher, 2004).

For these reasons, together with its low price, *methanol* is today the most used reactant for the biodiesel production.

From here on, most of the consideration that will be made in this work will be related to the reaction using methanol since it will be also the one used in the experimental part.

In Figure 1.8., the reaction for the formation of biodiesel from oil and methanol is shown.

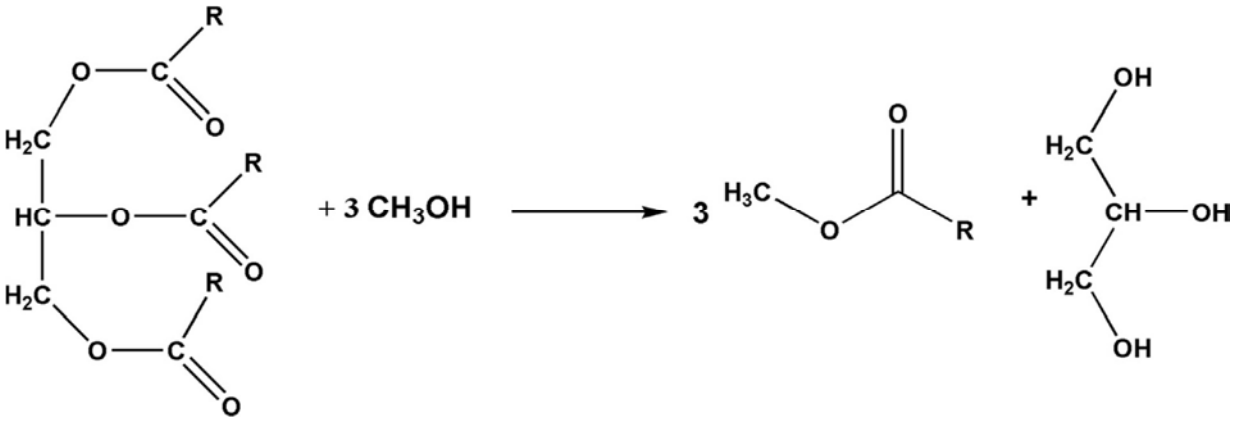


Figure 1.8. *Transesterification reaction with Methanol. R= Hydrocarbon chain (De Filippis et al., 1995)*

This is only a simplified reaction because mechanistically, the transesterification of triglycerides with methanol proceeds through three consecutive *reversible* reaction steps as shown in Figure 1.9. First the reaction between triglyceride and methanol results in FAME and diglyceride generation. As an intermediate compound, diglyceride reacts further with methanol to generate FAME and monoglyceride. Finally, the reaction between monoglyceride and methanol yields FAME and glycerol (Farobie and Matsumura, 2017).

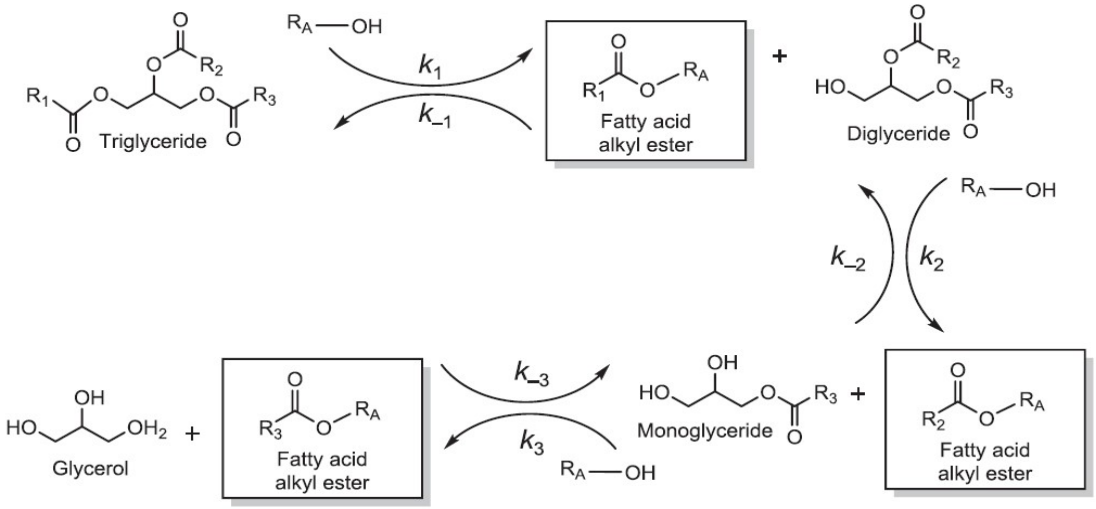


Figure 1.9. *Global reaction steps of biodiesel production under methanol and ethanol. Where: R1,R2,R3= hydrocarbon residues; RA=CH3 residue for methanol and C2H5 for ethanol (Farobie and Matsumura, 2017).*

As already mentioned, the reaction is reversible. This means that the temperature, pression, reaction time and removal of the product will have an important role on the final biodiesel yield. The influence of this parameters is studied in *Chapter 1.3.4.*

1.3.3 Side reactions

The presence of water and free fatty acids (FFA) in the feedstock or inside the reactor contribute to soap formation as the fatty acid chains are stripped form the triglycerides. The soap sequesters the alkali catalysts and inhibits the separation of the glycerol from the biodiesel. Excessive soap also contributes to the formation of emulsions when water washing is used at a later stage of the production process (Schumacher, 2004).
The reaction describing the saponification that occur when there is presence of water and or FFA in the feedstock are described in Figure 1.10 and Figure 1.11.

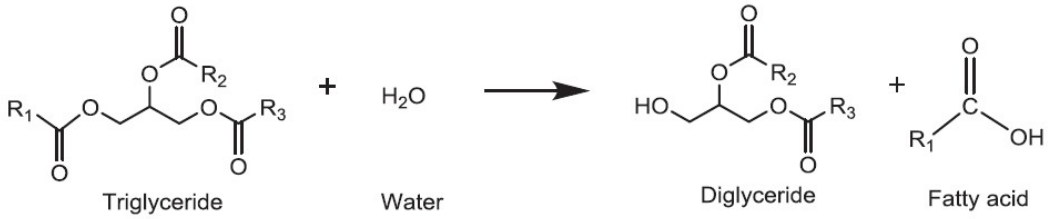


Figure 1.10. *Reaction of formation of FFA from water presence in feedstock (Farobie and Matsumura, 2017).*

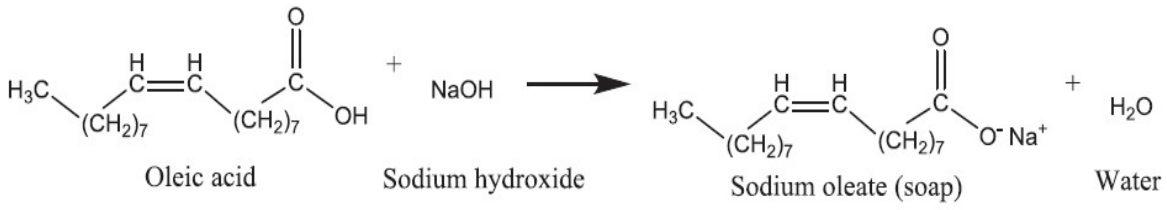


Figure 1.11. *Saponification from FFA and alkali catalysator (Farobie and Matsumura, 2017)*

In the first reaction, the FFA is formed due to the present of water. In the second one, the fatty acid (in the figure oleic acid) reacts with the catalyst to form the unwanted soap.

1.3.4 Operating conditions

The stoichiometric molar ratio between alcohol and oil is 3:1, but to help the reaction moving forward, the ratio industrially used start from a minimum of 6:1 to a maximum of 30:1, depending of the catalyst and alcohol used. In case of alkali catalysed reaction and using methanol like alcohol, the most advantageous reaction conditions correspond to a molar ratio alcohol:oil of 6:1, temperature of 60°C and atmospheric pressure. In general the reaction temperature has to be slightly below the boiling point of the alcohol (Knothe and Razon, 2017). Referring to the condition described above, in the case of the alkali catalysed reaction, the percentage of the catalyst to add in terms of catalyst mass over feedstock mass should be from 0.5% to 1%. The two alkali catalyst most commonly used are sodium methoxide (CH_3ONa) and sodium hydroxide (Verma and Sharma, 2016).

1.3.5 Raw materials

In a biodiesel production plant, the feedstock share of the total production cost can get as high as 75% for low production capacities and could get up to 90% when the production capacities increase. In most of the cases so, the share of the feedstock cost increases as the production scale get higher, making it less viable to scale up (Gebremariam and Marchetti, 2018).
Moreover, the choice of the feedstock to use in a biodiesel production plant is important not only in terms of profitability of the plant but also in terms of sustainability and eco-friendly.
The feedstock used in biodiesel production plant can be divided in three different categories:

- Edible Oil: This type of oil is the most used one because it is the cheap one. The major drawback is that this oil can also be used as food for people, so all the oil used for biodiesel is oil that is taken away from the world alimentation.
- Non-Edible Oil: this kind of oil can be seen as the future sources of biodiesel production. This include the oils that cannot be used in food industry, so they must be grown only for biodiesel production purposes and this usually increases their price. In some cases, they can be produced in areas where the edible oil cannot: road sides, irrigation canals and boundaries of cultivated areas.
- Waste Oil: In this category waste oil coming from the food frying factory and waste animals' fat are included. The advantage of this kind of feedstock is that these oils are cheap, and they are a waste for a lot of industries. The disadvantage is that this oil must first be purified. This process requires additional work and increases the total cost of production.

In Table 1.2., some of the feedstock belonging to the three categories just described are shown together with the percentage of oil content.

Table 1.2. *Different sources of oil for the production of biodiesel (Ambat, Srivastava and Sillanpää, 2018) [Adapted]*

Edible oils	Oil content (%)	Non edible oils	Oil content (%)	Animal Fat & other sources	Oil content (%)
Sunflower oil	25–35	Jatropha oil	30–40	Mutton Fat	
Soybean oil	15–20	Chinese tallow seed oil (stillingia oil)	44.15	Broiler chicken waste	
Rapeseed oil	38–46	Karanja(Pongamia pinnata oil)	27–39	Algae oil	
Peanut oil	45–55	Neem oil	20–30	Waste cooking oil	
Olive oil	45–70			Microbial oils	
Canola oil	40–45	Castor oil	45–50	Waste fish oil	
Palm oil	30–60	Sylbum marianum oil		Micro algae	30–70
Coconut oil	63–65	Rubber seed oil	53.74–68.35	Pine and kapok oil	
Mustard oil					
Linseed oil	40–44				

In most of the cases, the type of oil used for the biodiesel production is related to the type of cultivation that surround the area, due to the faster supplying and to the costs. For example, soybean oil serves as one of the major sources of biodiesel in the United States whereas in the tropical countries for the production is used palm oil and in Europe rapeseed oil.
The oil content of the culture shown in Table 1.2., is not the only characteristic of the oil that should be considered. Every oil has a different quantitative component of fatty acids.

Depending on triglyceride source, the biodiesel produced will have different carbon chain length with different saturation degree. This will bring to a change of the property of the biodiesel like viscosity, cetane number, cold flow and oxidative stability. In table 1.3. the percentage of fatty acid produced from the different oil feedstock is presented.

Table 1.3. *Compositions of the biodiesels in mass percentage; Biofuels by letters (S = methyl soy, R = methyl rapeseed, P = methyl palm, SR = soy + rapeseed) (Prieto et al., 2015). [Adapted]*

FAME	S	R	P	SR	PR	SP	SRP
C10	0	0.01	0.03	0	0.02	0.01	0.01
C12	0	0.04	0.24	0.03	0.2	0.18	0.14
C14	0.07	0.07	0.57	0.09	0.54	0.01	0.38
C16	10.76	5.22	42.45	8.9	23.09	25.56	18.97
C16:1	0.07	0.2	0.13	0.15	0.17	0.11	0.14
C17:0	0	0	0	0	0	0	0
C18	3.94	1.62	4.02	2.76	3.02	4.04	3.28
C18:1	22.96	62.11	41.92	41.82	52.92	33.13	42.51
C18:2	53.53	21.07	9.8	37.51	15.47	31.72	27.93
C18:3	7.02	6.95	0.09	7.02	3.08	3.58	4.66
C20	0.38	0.6	0.36	0.46	0.49	0.39	0.45
C20:1	0.23	1.35	0.15	0.68	0.67	0.2	0.52
C22	0.8	0.35	0.09	0.46	0.24	0.32	0.33
C22:1	0.24	0.19	0	0.12	0.09	0.12	0.14
C24	0	0.22	0.15	0	0	0.63	0.53
DU	144.6	120.0	62.0	131.8	91.0	104.2	108.5
$\rho_{m,\mathrm{GVOL}}^{298}$	3.0037	2.9721	3.0652	2.9929	3.0198	3.0277	3.0114
M	292.77	295.08	284.32	293.43	289.49	289.20	290.86
T_{min}	283.15	283.15	283.15	283.15	283.15	283.15	283.15
T_{max}	333.15	333.15	333.15	333.15	333.15	333.15	333.15
p_{min}	0.1	0.1	0.1	0.1	0.1	0.1	0.1
p_{max}	45.0	45.0	45.0	45.0	45.0	45.0	45.0
ρ_{min}	857.1	855.2	848.2	854.9	849.7	850.8	851.6
ρ_{max}	916.0	913.8	907.4	913.6	909.1	909.9	911.0
EoS	Tait	Tait	Tait	Tait	Tait	Tait	Tait
FAME	N9	N17	N19	N20	N21	N23	PCS

The free fatty acid (FFA) content of the feedstock is as important as the water content when is time to choose it. The two in fact can lead to saponification inside the reactor during the transesterification reaction.

In the case of feedstocks with high quantity of FFA, during the production of biodiesel there could some saponification. In this case, the operative conditions will have to be choose more precisely.

Animal waste and brown grease waste cooking oils are the two more difficult and expensive oil to treat. Therefore, they are usually mixed with other kind of oil and used only in small percentages.

1.3.6 Mass transfer

Because of the differences between the density of the alcohol and the oil, two different liquid phases will appear inside the reactor. In order to react the two phases must be in strict contact with each other. Thus, they are strongly mixed, and this generate the formation of an emulsion inside the reactor with small drops of alcohol inside the denser phase.
In literature are present five different types of techniques that can be used for the transfer of mass from one liquid phase to the other: Hydrodynamic cavitation, Ultrasound cavitation, Microwave, Co-solvencies and Mechanical stirring.
Hydrodynamic cavitation is rather promising: it consists in producing cavities inside the phases, which are effective in emulsification and results in excellent interfacial oil-methanol contact. This bring to the elimination of the mass transfer resistance and significantly enhance the conversion rate. Ultrasound cavitation works more or less in the same way, using ultrasounds.
Another different approach to eliminate the mass transfer limitation is the use of a co-solvent to improve the miscibility of methanol and alcohol. Some of the solvent, used in the reaction are tetrahydrofuran, dimethyl ether and methyl tert-butyl ether.
Using the co solvent the two phases become one, the contact between the two reactants is increased and the overall reaction rate can be enhanced (Knothe and Razon, 2017).
However, the most used mixing method is the mechanical stirring. It can, in fact, offer a good and cheap mass transfer between the phases (Chuah *et al.*, 2017). The mechanical stirring can be achieved using a common paddle-type impeller or more complex designed reactor like micro channel reactors, slit channel reactors, oscillatory flow reactors and rotating tube reactors.

1.3.7 Catalysts

In order to operate the transesterification reaction in acceptable operating conditions and with a profitable yield, a catalyst is needed. The catalysts currently used are grouped into three categories as homogeneous, heterogeneous and enzymes. Various catalytic trends in Biodiesel production is described in Figure 1.12.
The most preferred catalysts used to produce biodiesel are *homogeneous catalyst* as they are simple to use and require less time for the completion of the reaction. Another advantage related to this kind of catalysts is that they require mild operation conditions for the reaction to take place. The main disadvantage is that they generate a large amount of wastewater, in fact the FAMEs needs to be washed after the completion of the reaction with clean water in order to remove the catalyst.
Homogeneous catalyst is divided again between alkali and acid based.

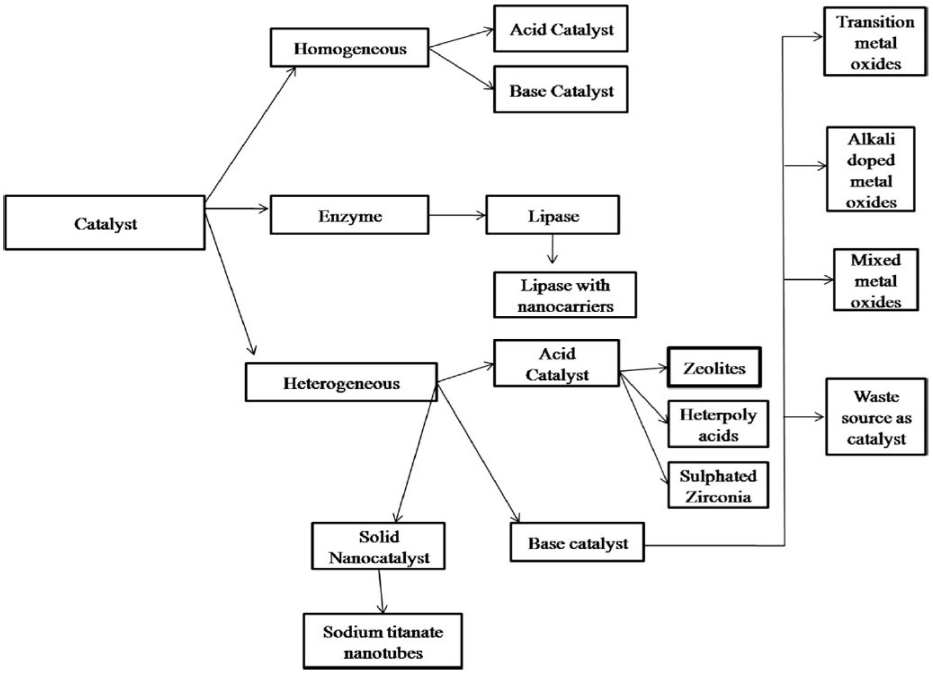


Figure 1.12. *Catalyst trend in biodiesel production (Baskar and Aiswarya, 2016)*

The *Alkali based* catalyst seem 4000 times faster than acid one and the most used are: NaOH, KOH, $NaOCH_3$ charged at 1% w_{cat}/w_{oil} for reaction at 65°C and alkali metal alkoxides in more difficult conditions (Baskar and Aiswarya, 2016).

If the oil has high free fatty acid content and more water than the normal, the *Acid catalyst* transesterification is more suitable. The acid catalyst normally used are Bronsted acid like sulfuric acid, phosphoric acid, hydrochloric acid or organic sulfonic acid (Meher, Vidya Sagar and Naik, 2006). However, sometimes also $AlCl_3$ and $ZnCl_2$ were used, but it results that the water content of the oil deactivates this kind of catalysts. The main disadvantages of the acid catalyst are the possibility of corrosion of the plant and the lower yield of the product in respect to the alkali one's (Baskar and Aiswarya, 2016).

The *Enzymatic catalyst* like lipase can effectively catalyse the transesterification of triglycerides in either aqueous or non-aqueous systems, which can overcome the problems mentioned above. Also the free fatty acid in the oils can be converted to products and the by-product, glycerol can be removed without any complexity (Fukuda, Kond and Noda, 2001). The only problem of the enzymatic lipase catalyst is the price. The production cost using this catalyst is significantly greater than the one of an alkaline catalyst.

The *Heterogeneous catalyst* is known to be a solid catalyst able to reach good yield in convenient conditions for the commercial production of biodiesel at industrial scale. They have a lot of advantages in respect to the homogeneous one like: they are tolerant with high fatty acid oils and high-water contents, they can be recycled many times and the separation is very simple. Also if they form a third phase with the alcohol and oil, this limitation can be solved by

using structure promoters or catalyst supports which can provide more specific area and pore for active species with the drawback of the increase of the costs (Baskar and Aiswarya, 2016). Heterogenous catalysts can be derived from a variety of different biomass like mollusc shells, egg shells, ashes, rocks and waste resources. However, sometimes this kind of materials present problems related with the satisfaction of the products specification's standards. This problem, the mass transfer limitations, the leaching of the solution and their production cost are the four main obstacle that are stopping industrial usage of this type of catalyst in biodiesel production (Knothe and Razon, 2017).

1.3.8 Biodiesel Production

The simplified scheme of the biodiesel production process commonly practiced in industry is shown in Figure 1.13. As already mentioned in the previous chapter, the two reagents for the transesterification reaction are the oil which is more convenient for the industry and the alcohol pre-mixed with the catalyst.

The simplest way to conduct the reaction is the use of a batch, stirred tank reactor. The oil is usually charged before and the alcohol, premixed with the catalyst, afterward. The usual ratio between alcohol triglyceride is 6:1 (molar) and the other operating condition are the temperature, usually ranged between 298K and 358K and the atmospheric pressure. The catalyst loading range can be from 0.3% to 1.5% and the reaction time ranges from 20 minutes to 1 hour.

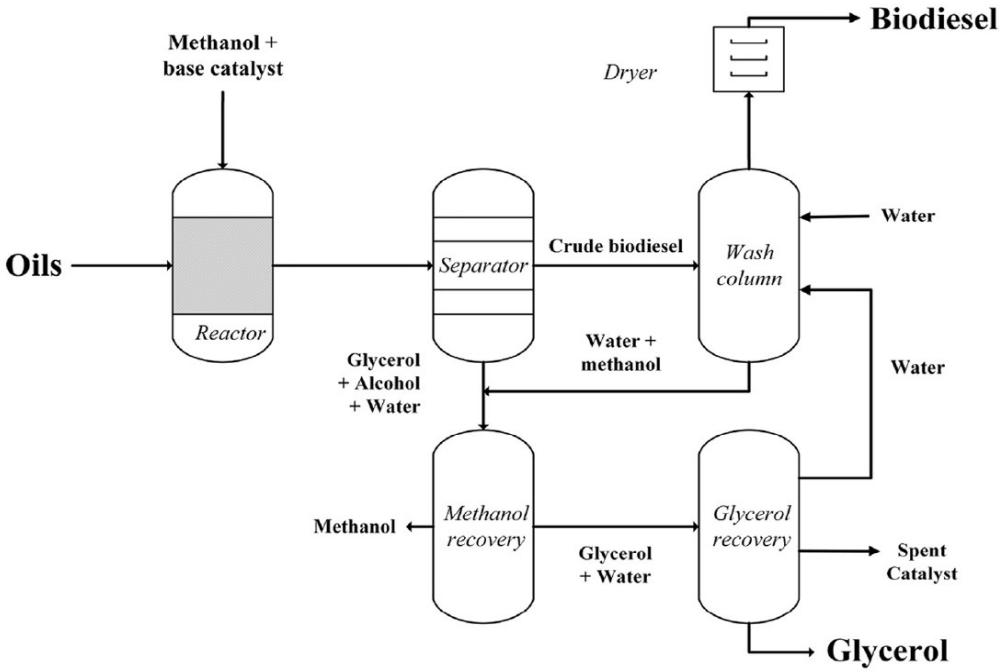


Figure 1.13. *Biodiesel production process (Knothe and Razon, 2017)*

The reactor may be sealed or equipped with a reflux condenser in order to prevent the loss of alcohol. The reactor is usually agitated by an impeller.
In some cases, during the course of the reaction, the agitation is stopped for some minutes. This technique allow a first separation of the glycerol and the esters inside the reactor.
After the reaction, the products are pumped into a settling vessel or are separated using a centrifuge which separates the heavy glycerol from the light esters. The non-reacted alcohol can be washed out from both stream using a washing column, a flash unit or an evaporator and can be recover and reused. The esters are neutralized, washed gently using slightly warm acid water (to remove the residual salts) dried and sent to storage.
The glycerol stream with water is then neutralised and sent to the recovery and refining unit, in order to separate the water that can be reused with the glycerol that now can be stored.
In some cases, for example when the transesterification in performed using alkaline, acid, or enzyme catalyst, other reactor can be adopted. There are several processes that use intense mixing, either from pumps or motionless mixers, to initiate the transesterification reaction. A popular variation of the batch process is the use of continuous stirred tank reactors in series. The reaction mixtures move inside the reactors in a continuous plug with little mixing in the axial direction. The result is a short residence time for the completion of the reaction (Ayhan Demirbas, 2008).

1.3.9 Biodiesel Standard

To be used by the engines commonly founds in the current market, biodiesel has to be mixed with the common petrodiesel. The two fuels, mixed together, form a blend. The blends that contain biodiesel are called with the B letter followed by the percentage of biodiesel present inside the blend while the remaining percentage is petrodiesel.
In order to ensure the biodiesel quality, Europe and USA developed two different standards called *EN14214* and *ASTM D6751* respectively. These two standards define the requirement for B100, the blend composed only by biodiesel. B100 that meet this standard could be used unblended in a diesel engine (if the engine has been adapted to operate on B100) or blended with petroleum diesel fuel.
Besides the general standard for biodiesel, there is also a standard that regulates the blending of fossil diesel with biodiesel.
According to the European standard *EN 590* "Automotive fuels, Diesel, Requirements and test methods" additions of up to 5 % volume biodiesel (FAME) to diesel fuel are permissible without labelling. Higher blends, always below B100 (100% biodiesel), may be sold, but have to be labelled accordingly (Rutz and Janssen, 2006).
The European general standard EN24214 shown in Table 1.4. replaced several old national standards in different European countries. Stricter standard may exist in other countries.

Table 1.4. *EN 14214 biodiesel standard (Rutz and Janssen, 2006)*

Property	Unit	Minimum	Maximum	Test Method
Ester Content	% (m/m)	96.5	-	EN 14103
Density at 15 °C	kg/m³	860	900	EN ISO 12185
Kin. Viscosity at 40 °C	mm2/s	3.5	5.0	EN ISO 310
Flash Point	°C	Above 101	-	ISO / CD 3679
Sulfur Content	mg/Kg	-	10	-
Carbon Residue (10% Bottoms)	% (m/m)	-	0.3	EN ISO 10370
Cetane Number	-	51.0	-	EN ISO 5165
Sulphated Ash Content	% (m/m)	-	0.02	ISO 3987
Water Content	mg/Kg	-	500	EN ISO 12937
Total Contamination	mg/Kg	-	24	EN 12662
Oxidation Stability, 110 °C	hours	6		pr EN 14112
Acid Value	mg OH/g	-	0.5	pr EN 14104
Iodine Value	-	-	120	pr EN 14111
Linolenic acid methyl ester	% (m/m)	-	12	pr EN 14103
Polyunsaturated (≥ 4 double bonds) methyl esters	% (m/m)	-	1	-
Methanol Content	% (m/m)	-	0.2	pr EN 14110
Monoglyceride Content	% (m/m)	-	0.8	pr EN 14105
Diglyceride Content	% (m/m)	-	0.2	pr EN 14105
Triglyceride Content	% (m/m)	-	0.2	pr EN 14105
Free Glycerol	% (m/m)	-	0.02	pr EN 14105 pr EN 14106
Total Glycerol	% (m/m)	-	0.25	pr EN 14105
Alkaline Metals (Na + K)	mg/Kg	-	5	pr EN 14108
Phosphorus Content	mg/Kg	-	10	pr EN 14107

1.4 Monitoring of biodiesel: Analytical techniques

During the transesterification reaction for the biodiesel production, various compounds will form, as MG, DG, FAME, glycerol, from the reactants as alcohol, oil and catalyst.
Knowing the quantity of this compounds allow to understand, not only the quality of Biodiesel produced, but also the progress state of the reaction. In order to know their quantity inside the reactor or in the product, the reacting mixture has to be analysed.
Among all the analytical techniques chromatography and spectroscopy are the most convalidated ones. These techniques are used for the characterization of the final product. However they represent a big investment in term of money. Moreover, they have the drawbacks that they are not on-line, and they need time from the sample taking to the completion of analysis. This does not allow to have a fast knowledge of the reaction rate and yield (Monteiro *et al.*, 2008).
In the last years the studies started focusing on the physical property of Biodiesel and on how they change during the reaction. Because of the variety of the feedstock, these properties change form production to production but, knowing their typical behaviour inside the reactor, it is possible to have an idea on the characteristics of the biodiesel produced and the progress of the reaction without sampling.
The physical properties usually analysed are *density*, *viscosity* and *speed of sound*. The analysis of these properties can be done off and on-line, allowing the fast determination of the states of the current production (De Filippis *et al.*, 1995).
In the next pages some of the newest and most promising analysis techniques are given, describing the property that is analysed and the analytical method which is used.

1.4.1 Refractive Index

During the Biodiesel production, the refractive index inside the reactor tend to decrease because of the different refractive index of the products and the reactants as can be seen in Table 1.5.

Table 1.5. *Physical property of compound indicated* (Zabala *et al.*, 2014) *[Adapted]*

	Density (ρ, g/ml)			Refractive index (n_D)		Speed of sound (u_s, m/s)		
	20 °C	25 °C	50 °C	20 °C	50 °C	20 °C	40 °C	50 °C
Sunflower oil	0.923		0.902	1.475	1.455	1464		1395
FAMEs	0.882		0.863	1.456	1.436	1406		1328
Methyl linoleate		0.870		1.4615[b]			1348[c]	
Methyl oleate		0.882		1.4521[b]			1338[c]	
Methyl stearate				1.4365[b]			1320[d]	1299[d]
Methyl palmitate				1.4332[b]			1318[c]	1285[d]
Methanol	0.790		0.764	1.329	1.311	1200		1180
Glycerol	1.262		1.244	1.474	1.458	1921		1874

[a] Data at 1 atm.
[b] Data from Gouw and Vlugter [29].
[c] Data from Gouw and Vlugter [30].
[d] Data from Ott et al. [31].

The samples taken out from the reactor to perform the off-line refractive index analysis were first centrifugates in order to separate the alcoholic and oil phases. Then samples were obtained from each phase to perform the n_D (Refractive index) measurement.
To confirm the analysis, samples were taken from the reactor and they were first neutralized with acetic acid, diluted with tetrahydrofuran and analysed with size-exclusion chromatography (SEC).
Using this method, a direct relation was founded between the conversion of the biodiesel and the increasing of the refracting index of the samples.
This confirm that is possible to understand the conversion of FAMEs only analysing the refractive index of the sample. The drawback is that it is an off-line method and it seem difficult to use it on-line because of the difficulty of two phases separation and the flow instability. It was in fact easiest measure the change of n_D in the off-line alcoholic (Light) phase than in the oil one (Zabala *et al.*, 2014).

1.4.2 Speed of sound

The difference between the speed of sound *(u_s)* the species taking part on methanolysis reaction (see Table 1.5), suggest that monitoring can be made following the changing of this property.
Furthermore, the difference between the different speeds of sound in different oil with different fatty acid profile (number of carbon atoms and carbon-carbon double bond) is small and this suggested that the measurement of the u_s could be a good method for different kind of edible oil (McClements and Povey, 1992).
To confirm this theory, some analyses were performed on-line in a series of reactions with different oil/alcohol ratios and under different temperatures.
Also in this case, like the one in the previous chapter, the analysis of the property was confirmed by SEC analysis of samples taken from the different reactions.
The results confirmed that there was a linear correlation between the u_s and the conversion of the oil. The technique is very fast, accurate, sensitive and simple. It can be useful for process control and fundamental studies of the reaction kinetics (Zabala *et al.*, 2014).

1.4.3 MIR Mid-Infrared spectroscopy and partial least square model

The application of the mid-infrared spectroscopy and partial less square model as an analytical method for the quality control of biodiesel seem promising.
Due to a non-disclosure agreement the experimental details are not revealed but, the analytical technique seems to be easy to implement, quick and with low waste generation.
The online analysis seems possible, implying that the sampling of the biodiesel from the reactor is not needed and it can be used almost everywhere.

With this technique it seems possible to quantify MG, DG, TG, water, ashes, and glycerol content and the provided results are quite satisfactory when compared to conventional analytical methods.
The only drawback is that a model (partial less square) is needed (Bonato *et al.*, 2018).

1.4.4 Impedance measurement

The sensor used for the impedance measurement consist of two sets of interleaved electrodes separated by a gap. Between the two fingers the AC voltage generate an oscillating electric field which penetrate the material surrounding the sensor, in this case the reacting fluids. The dielectric properties (permittivity) of the fluids will influence the interaction between the last and the electromagnetic field applied. The sensor will detect changes in capacitance and electrical conductivity of the material under test. The ratio between the excitation voltage applied and the resulting current flow is the impedance (Z).
In the case of the transesterification reaction the sensor can describe the trend that the reaction takes only during the first period and help identify the steady state conditions. This can be useful to determine the moment where the mechanical stirring act inside the reactor, mixing the two reactants and also, identify when the reaction reaches an end. This can help to understand the moment when reducing the stirring power and the time to ending the reaction, in order to spare time and electrical power.
Furthermore, the sensor is easy to fabricate, has no moving parts and requires relative simple electronics (T *et al.*, 2014).

1.4.5 Low resolution/ Unilateral, Nuclear Magnetic Resonance

Performing the transesterification reaction, some samples are taken from the reactor, dilute in chloroform and analysed in the NMR. In the case of this study three different type on NMR are compared: High resolution, Low resolution and Unilateral NMR. All three shows very good results, allowing to follow the Biodiesel production during the time.
Low resolution and unilateral resolution NMR are fast, efficient, and non-destructive techniques used to monitor transesterification reactions. The high-resolution NMR uses expensive equipment and solvent. The low resolution unilateral are more suitable to measurement in industrial environment. The only disadvantage of the conventional Low resolution is that, with the present of alcohol in the mixtures the analysis is not very reliable. Although, a simple evaporation of the residual alcohol can solve the problem. The unilateral NMR seem to don't have this problem. This last techniques may be the best to be used to monitoring the reaction in situ (Cabeça *et al.*, 2011).

1.4.6 Analytical Balance

This type of analysis consists in the study of a known quantity of the reaction mixture, deposit it in an analytical balance and determine the exact weight. The formation of glycerol and of FAME inside the reactor changes the density of the liquids that will have a different weight in the balance.
The analysis is easy, really cheap and can be perform in real time.
The main drawbacks are that the system as to be sealed, in order to avoid the loss of alcohol and the sample taken must have always the same volume, in order to determine its changes. The analysis is not very precise and if the reactor is not well agitated, the methanol will change the density of the liquid in a non-linear way, but only when it is entering the mixture (TUBINO, *et al.*, 2018).

1.4.7 Ultrasound

Ultrasound is a mechanical wave that propagates in fluids at frequencies greater than 20kHz, i.e., out of the audible range for healthy humans.
This method consists in insert a sensor inside the reactor where the transesterification reaction is performed. The sensor emits an ultrasound that propagate and reflect in the medium and is successively receipted again by the sensor. The changing in the time to flight and the pulse amplitude of the signal define the fluid that is inside the reactor.
The ultrasound analysis has revealed that the velocity of propagation inside the reactor diminish with the increasing of biodiesel produced.
This method is remarkably simple to use and non-destructive. It can be use also online when the stirring is fixed, and the reaction kinetics is known: like in biodiesel production.
Though, the experiment must be carefully designed; the physical process that occurs between the transmission and receiving of the signal is complex. If the experiment is not well analysed and rightfully carried out the outcome of the ultrasound analysis could be of no technical usefulness (Baêsso *et al.*, 2018).

1.4.8 pH measurement

The pH is usually used to monitor the reaction in aqueous solution, but in this case, it can be used to determine the changing of basicity of the vegetable oil that react to biodiesel. The completion of the reaction is indicated by the exhaustion of the basicity inside the reactor due to the consumption of the OH^- present because of the catalyst and the alcohol.
Once the methanol and the catalyst are added to the reactor, the pH changes immediately from around 10 to almost 14, and slightly decrease till it reaches the stability. When the pH does not change anymore, it means that the steady state has been reached. Low pH indicates high conversion of biodiesel.

The pH measurement its a cheap and non-destructive method that can be taken continuously, in situ, to monitor the reaction progress. The pH value can be related with the reaction conversion in order to fit a kinetic model for different kind of feedstock (Clark *et al.*, 2013).

1.5 Reaction kinetics

The study of the kinetics for the transesterification reaction of the biodiesel production is still an open question. In the reports that can be found in literature the kinetic scheme is simplified and described in different ways as reversible/irreversible and, consecutive/non-consecutive. Different order were considered too: pseudo-first-order, pseudo-second-order or reaction rate order changing along the course of the reaction. (Tubino, Junior and Bauerfeldt, 2014)
To analyse the kinetic of the reaction, the first decision that should be made is to consider the main reaction only, or to divide the reaction and study all the steps that lead to the production of biodiesel. In the next pages, the two approaches are presented. To simplify the analysis, the side-reaction were not considered.

1.5.1 Main reaction

A simplification that can be made in order to analyse the kinetic of the transesterification reaction for the production of the biodiesel is to consider only reaction (1.1), which is the main reaction:

$$TG + 3\,M \leftrightarrow G + 3\,FAME \tag{1.1}$$

Where TG = Triglyceride, M = Methanol and G = glycerol.
The reaction is known to be endothermic and reversible and, thus, high temperature and excess of reactants will increase the yield.
In this study, as a simplification, the reaction will be assumed as irreversible.
The reaction rate of Eq. (1.1) in terms of changing of the triglycerides concentration C_{TG}^{a} methanol C_{M}^{b} is written as:

$$r = -\frac{d\,C_{TG}}{d\,t} = k_1\,C_{TG}^{a}\,C_{M}^{b} \tag{1.2}$$

Where k_1 is the kinetic constant of the reaction, a and b are orders relatives to TG and M respectively. This main reaction can be assumed to proceed like a first or second order reaction, but in both cases, it will be dependent only from the concentration of the vegetable oil. This can be explained by the fact that the methanol is in excess inside the reactor and that the reaction takes place in a heterogeneous environment. Thus Eq. (1.2) can be rewritten as:

$$r = -\frac{d\,C_{TG}}{d\,t} = k_1'\,C_{TG}^{a} \tag{1.3}$$

where $k_1' = k_1\,C_M^b$. If we consider a first-order reaction (Jain, Sharma and Rajvanshi, 2011), the exponent "*a"* will be set equal to 1, and Eq. (1.3) can be integrated as:

$$\int_{C_{TG}^0}^{C_{TG}^t} \frac{d\,C_{TG}}{C_{TG}} = -k_1' \int_0^t d\,t \tag{1.4}$$

Giving the result:

$$ln\frac{{C_{TG}}^t}{C_{TG}^0} = -k_1'\,t \tag{1.5}$$

If we consider the reaction as a second order reaction, a=2, and Eq. (1.3) will be integrated as:

$$\int_{C_{TG}^0}^{C_{TG}^t} \frac{d\,C_{TG}}{C_{TG}^2} = -k_1' \int_0^t d\,t \tag{1.6}$$

Resulting:

$$\frac{1}{C_{TG}^t} - \frac{1}{{C_{TG}}^0} = k_1'\,t \tag{1.7}$$

This left member will describe the changing of concentrations as a linear function of the time.

1.5.2 Sequence of reactions

In order to perform a more complete and deep analysis of the reaction's kinetics, the main reaction is not enough. As explained in Chapter 1.3.3, the reaction to produce biodiesel is divided in three different reversible reactions (1.8) to (1.10) that, at summation will give Eq. (1.1).

$$TG + M \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} DG + FAME \tag{1.8}$$

$$DG + M \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} MG + FAME \tag{1.9}$$

$$MG + M \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} G + FAME \tag{1.10}$$

Studies on these reactions were done extensively in relation of different feedstocks. In most of the cases the results from this studies suggested that the reactions behave like a second order reaction (Ellis *et al.*, 2008; Richard, Thiebaud-Roux and Prat, 2013; Tubino, Junior and Bauerfeldt, 2014).

In the case of the rapeseed oil, the feedstock that will be used in the practical part of this thesis, the studies performed using three reactions set that the second order is a realistic model to describe the kinetics (Komers *et al.*, 2002).

The simulation of the behaviours of the reactions (1.8) to (1.10) is shown in Figure 1.14. This simulation has the scope to display how the reactants and the products behave inside the reactor and can give an idea of the kinetic of the reactions.

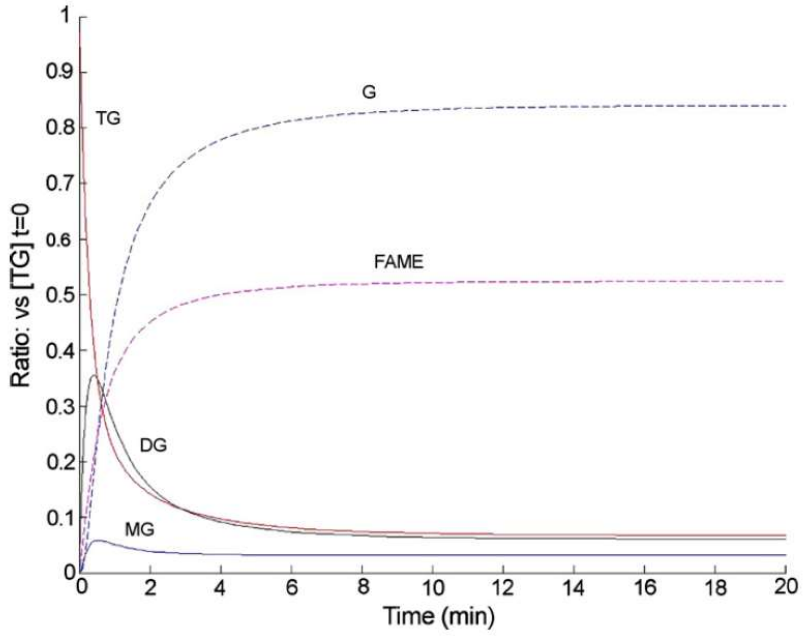


Figure 1.14. *Simulated concentration profile of glycerol (G), tri-glyceride (TG), di-glyceride (DG), mono-glyceride (MG) and FAME during transesterification (Ellis* et al.*, 2008).*

Chapter 2

Materials and methods

The main task of this study is the monitoring of the transesterification reaction through the change of the viscosity of the fluid inside the reactor.
During the monitoring of the viscosity inside the reactor, the same mixture is sampled and analysed with HPLC method. The latter is then used to determine the conversion and the yield of the reaction, in order to define the best reaction time and conditions.
In this chapter are describes the materials, methods, instrumentations and analytical techniques used to perform the practical part of this thesis and to analyse the products.
All the tests were performed in a laboratorial environment respecting all the substances' safety recommendations and following the advices regarding the laboratory work. (Appendix A)

2.1 Viscosity

All fluids have some resistances to movement when stress is applied. This resistance is called viscosity and its value define how much a fluid is viscous. It can be said that viscosity quantifies a fluid's resistance to flow.
Viscosity is expressed in two forms: as absolute (or dynamic) viscosity (μ) and as kinematic viscosity (υ).
The kinematic viscosity requires knowledge of the density of the liquid (ρ) at given temperature and pressure and is defined as:

$$\upsilon = \frac{\mu}{\rho} \tag{2.1}$$

In this study, only the dynamic viscosity μ will be considered (Viswanath, D.S., Ghosh, T., Prasad, D.H.L., Dutt, N.V.K., Rani, 2017).

The unit of measure of dynamic viscosity in the SI system are Pa·s or N·s /m^2. It is not uncommon to find this property also expressed within the cgs units of measure that is the cP (centipoise).

2.1.1 Newtonian and non-Newtonian fluids

When the viscosity of a liquid remains constant and it is independent of the applied shear stress, such liquid is termed as a Newtonian liquid.
In the case of the non-Newtonian liquids, viscosity depends on time and on the applied shear force, this behaviour is shown in Figure 2.1.

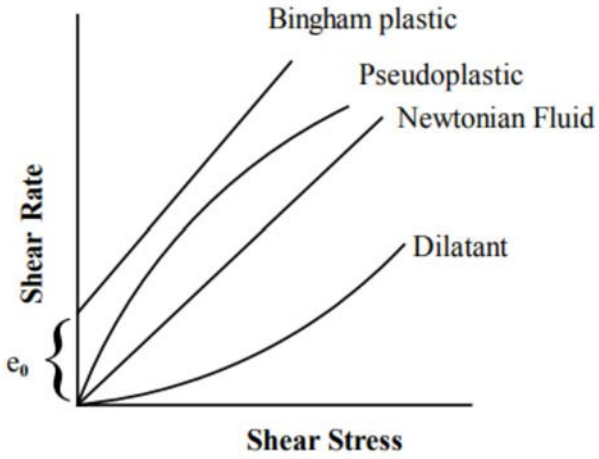
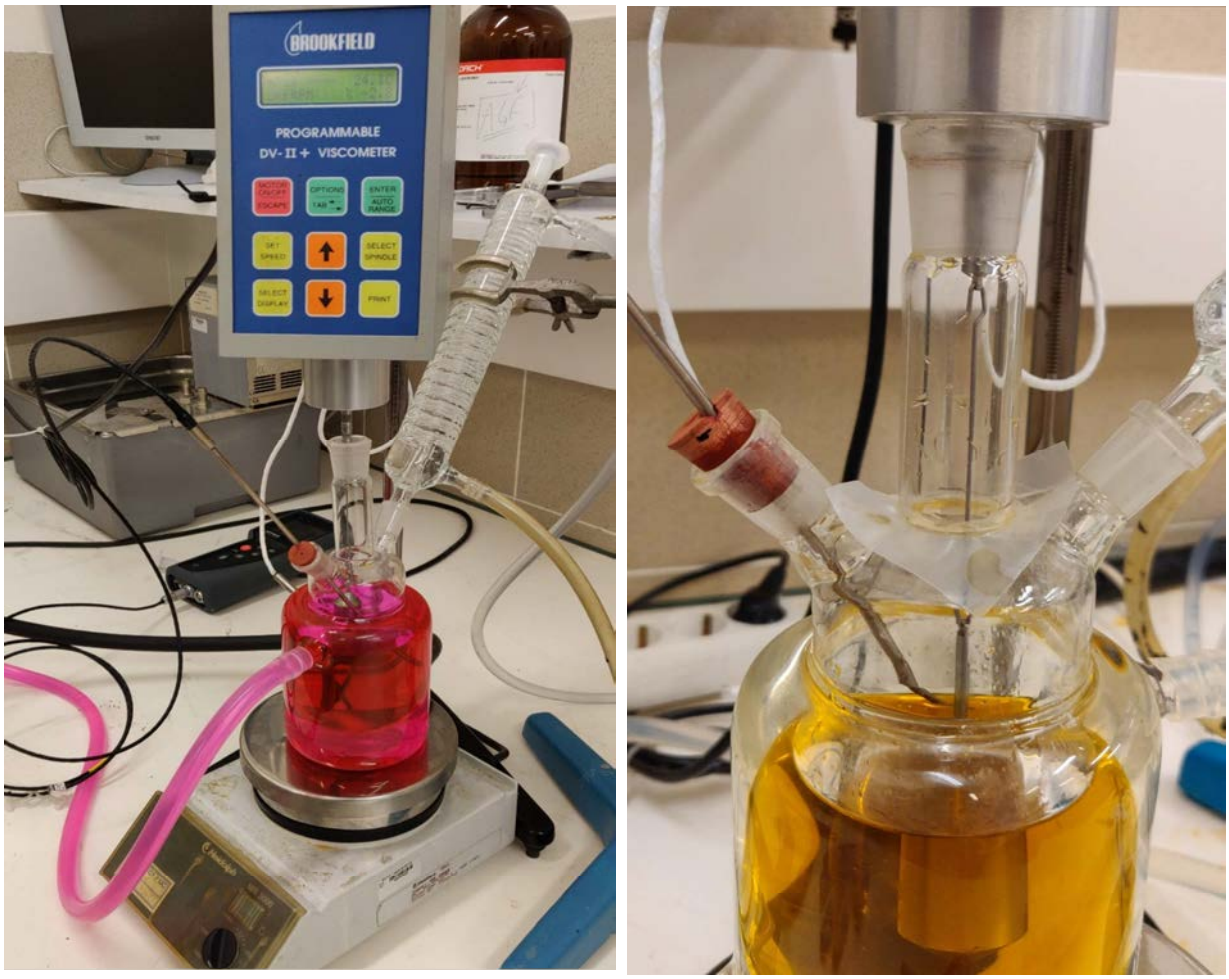


Figure 2.1. *Fluid types (Viswanath, D.S., Ghosh, T., Prasad, D.H.L., Dutt, N.V.K., Rani, 2017) [Adapted]*

The pseudoplastic behaviour is the most common and it is characterized with the decreasing viscosity with the increasing shear rate. The dilatant one instead, displays increasing viscosity with an increase shear rate. The Bingham plastic is directly proportional to the shearing stress in excess with the yield stress e_0 that increase its shear stress behaviour by a constant value in respect to the Newtonian's one (Viswanath, D.S., Ghosh, T., Prasad, D.H.L., Dutt, N.V.K., Rani, 2017).
In order to understand if a fluid is Newtonian or not it is necessary to measure its viscosity while applying different shear stress. If the fluid property will behave proportionally with the shear stress, that fluid will be consider Newtonian. In the other case, the response of the fluid to the shear stress will define its non-Newtonian family.

2.2 Experimental setup

The aim of this study is to monitor the viscosity inside the reactor where the transesterification reaction take place. A 250 ml jacketed reactor was modified, cutting the upper part, allowing the spindle to enter to measure the viscosity inside the reactor. The reactor with all devices is shown in Fig. 2.2.a, and the sealing devices of the reactor are shown more in details in Figure 2.2.b.



a b

Figure 2.2. ***a****. Experimental Setup;* ***b****. Details of the reactor sealing*

The reactor is agitated with a magnetic stirrer and heated up by a *F12-ED thermostatic* bath with ethylene glycol. The temperature inside the reactor is monitored continuously by inserting the thermal probe (an electronic thermometer *Isotech TTI-10*) through a hole made on purpose in a rubber plug. The viscosity is measured using a *Brookfield programmable viscometer DV-II +* equipped with a S18 spindle, properly choose for the predicted range of viscosity (see Chapter 2.5).

The spindle is placed inside the reactor, in order to measure the viscosity on-line during the reaction. Since methanol is present, the reactor has been provided with a condenser on one of

the ends and the viscometer is connected to the reactor with a special glass tube made on porpoise for this experiment. It is in fact important to avoid the losses of methanol during the reaction in order to have better conversion of the TGs.

2.3 Materials

The reagents and chemical compounds used in the practical experiments and during the analyses are summarized in Table 2.1, together with their CAS number, the origin and the purity.

Table 2.1 *Reagents used in practical experiments and analyses*

Compound	CAS	Origin	Purity
Rapeseed Oil	8002-13-9	PRIO	-
Methanol	67-56-1	Fischer chemicals	99.99%
Glycerol	56-81-5	Fischer chemicals	99.95%
Sodium Methoxide	124-41-4	Alfa Aesar	98%
n-Hexane	110-54-3	Carlo Erba Reag.	98.5%
2-propanol	67-63-0	Fischer chemicals	99.5%
Acetonitrile	75-05-8	Fischer chemicals	99.9%

The safety data sheets of this compounds are resumed in the APPENDIX A together with the safety behaviours to maintain when these compounds are in use.

2.4 Transesterification reaction

The feedstock used to perform the transesterification reaction is the Rapeseed oil which is used in the industrial plant of the company PRIO, associated to this project. A picture of the initial state of the oil is shown in Figure 2.3.

The quantity of water inside the rapeseed oil was determined by Karl Fischer and result to be lower than 0.17% of the mass of the sample.

The other reagent, methanol, is the alcohol that, is responsible of the methylic group added during the reaction to produce the methyl esters FAMEs. The catalyst used is sodium methoxide, a homogeneous alkali-based catalyst usually faster and less dangerous than the acid one.

Figure 2.3. *Rapeseed oil from PRIO, used in the transesterification the reactions.*

The experiments were performed changing the alcohol/oil ratio from 4:1 mol/mol to 6:1 mol/mol and the temperature from 40 °C to 50 °C. Also, the same environmental conditions were kept during all the experiments, in order to have comparable results.
For each experiment 150 ml of rapeseed oil were used. The quantity of alcohol and catalyst are calculated as follows. With the fixed volume of oil (V_{oil}), the mass of oil m_{oil} is calculated through the density ρ_{oil} as:

$$m_{oil} = V_{oil} \times \rho_{oil} \tag{2.2}$$

The quantity of oil, in mol, is calculated from the molecular weight of the latter. Table 2.2 shows the mole fraction x_i of the triglycerides present in quantity higher than 1.5% inside the commercial rapeseed oil and their respective molecular weights M_i. The fractions are found in Table 1.3 and the molecular weight can be easily calculated knowing the chemical structure of the TGs.

Table 2.2. *Mole fraction and molecular weight of the main TGs components of rapeseed oil*

	C_{16}^{0}	C_{18}^{0}	C_{18}^{1}	C_{18}^{2}	C_{18}^{3}
Mole fraction x_i	0.052	0.016	0.621	0.211	0.07
Mol. Weigh M_i[g/mol]	256.42	284.48	282.46	280.45	278.43

With this value, and considering the molecular structure of a triglyceride, the Eq. (2.3) allows to calculate the molecular weight of the rapeseed oil.

$$M_{oil} = 3 \times \sum x_i \times M_i + 38.05 \tag{2.3}$$

Is now possible to calculate the number of mol of oil n_{oil} :

$$n_{oil} = \frac{m_{oil}}{M_{oil}} \tag{2.4}$$

Depending on the defined molar ratio (r) between alcohol and oil, the number of mol of alcohol are calculated:

$$n_{Alcohol} = r \times n_{oil} \tag{2.5}$$

Once the moles of alcohol needed are found, adjusting Eq. (2.4) and Eq. (2.2) and using the alcohol properties instead of the oil one's, it is possible to calculate the volume or the mass of alcohol to perform the reaction.
In this thesis the two molar ratios (*r)* considered to perform the experiment were 4 and 6. The other data used to make the calculations are shown in Table 2.3.

Table *2.3.* *Densities and molar weight of the compounds used.*

Rapeseed oil		Methanol	
M_{oil}	ρ_{oil}	$M_{alcohol}$	$\rho_{alcohol}$
851	0.9189	32.02	0.785

The mass of the catalyst m_{cat} to be used in the reactions is calculated as:

$$m_{cat} = m_{oil} \times \frac{\%_{cat}}{100} \tag{2.6}$$

Following the guideline described in chapter 1.3.7 the quantity of catalyst used to perform the experiment was decided to be 1% of the mass of the oil and the reaction time varying from 60 min to 70 min.

2.5 Spindle Selection

The selection of which spindle to use is based on the viscosity of the fluid that must be analyse. Each spindle has a range of viscosity where the results are considered as acceptable.

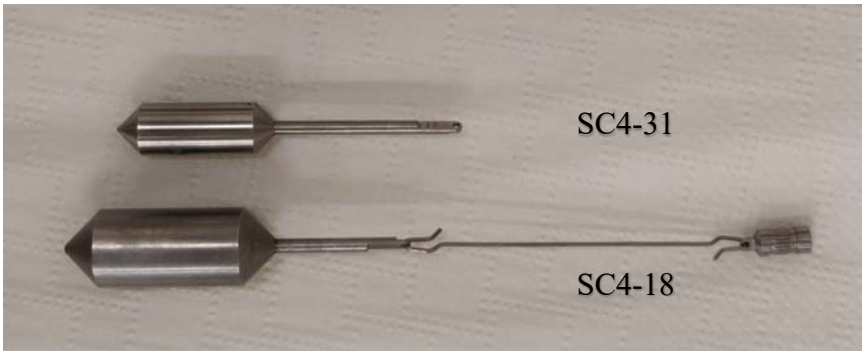


Figure 2.4. *Spindle S-18 and S-31*

The two spindles used with the Brookfield viscometer and the thermosel are shown in Figure 2.4. The different ranges of viscosity in which the two spindles can be used are found in the viscometer manual and they are reported in Table 2.4 within the standard sample size, the diameter, the length and the uncertainty factor (LV).

Table 2.4. *Characteristic values of spindles S-18 and S-31 from Brookfield manual.*

Spindle	Viscosity range [cP]	Sample [ml]	Diameter [cm]	Length [cm]	LV
SC4-31	15-300 000	10	0.463	0.990	300/N*
SC4-18	1.5-30 000	8	0.688	1.249	30/N*

While LV defines the uncertainty of the measured performed, N* is the spindle velocity expressed in RPM. When LV is multiplied per 100 it defines the value of the maximum measurable viscosity at that spindle velocity. Considering all this information and the predicted viscosity of the mixture founded in the previous works (De Filippis *et al.*, 1995; Ellis *et al.*, 2008)(Ellis *et al.*, 2008), the more suitable spindle to measure oil and biodiesel at the temperature which the reactions are performed is the SC4-18. This Spindle is in fact the more precise and rigorous in the range of viscosity that the mixture will change during the reactions.

2.6 Calibration curves

Because of the different shear stress applied to a moving fluid, the viscosity of the liquid measured inside the agitated reactor will be lower than its real one. In order to have the real value of the viscosity, the measure must be performed in an off-line standard environment. The standard off-line environment must have a defined geometry, with known dimensions, define volume of liquid and controlled temperature. In the case of the Brookfield viscometer, the standard is a steel cylinder, that is furnished alongside with the instrument of measure. To keep a constant temperature during the experiments the *Brookfield thermosel model 74R* is used. The complete instrumentation used to define the calibration curve is shown in Figure 2.5.

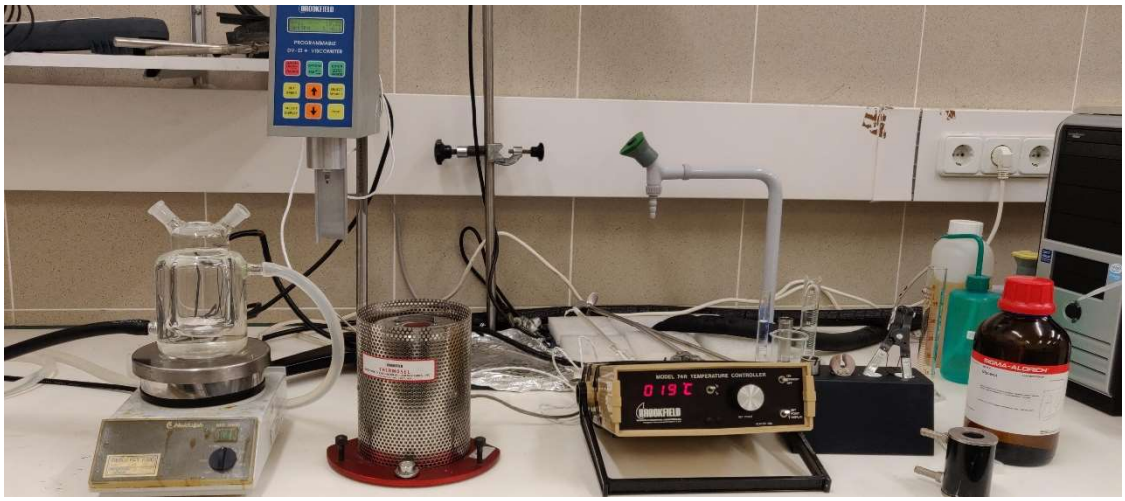


Figure 2.5. *Thermosel, Viscometer and reactor used to calculate the calibration curves.*

The calibration curves define a relation of the viscosity variation when it is analysed inside the reactor, with a mild agitation, and when it is measured using the standard off-line instrumentation. To perform the experiment and compare the apparent and real viscosity's values, the reactor is filled with 209 ml of glycerol (99.95% Fischer Chemicals) and 45 ml of water (Milli-Q). The viscosity of this mixture will be inside the range needed for the future experiments with biodiesel.

After measuring the viscosity of the mixture inside the reactor, at the temperatures of 30°C, 40 °C and 50°C and at different spindle velocities, an 8 ml sample is taken from the reactor and analysed inside the off-line standard environment at the same temperatures and velocities. The volume of fluid extracted from the reactor is replaced with pure water in order to change the composition inside the reactor and decrease the viscosity of the mixture. Measuring the viscosity of the mixture inside the reactor and the viscosity of the same mixture inside the standard allowed to draw the calibration curve that relate its apparent viscosity within the real one. This calibration curve will be useful in the experiment, allowing to define the real viscosity of the mixture during the monitoring of the reaction without sampling and without passing again through the analysis using an off-line standard environment.

Once the real behaviour will be known, it will be possible to use the results of this work also in different environments and conditions. The only need will be the draw of the calibration curve specific for the new system.
In other to understand at the best the behaviour of the mixture, three curves were defined at three different temperature: 30°C, 40°C and 50 °C.

2.7 HPLC analysis methods

During the experiments, samples were extracted from the reactor at different times and successively weighted and quenched with n-Hexane at 0°C. The sampling allowed to follow the conversion of the reagents during the viscosity change monitoring and the quenching was necessary to stop the reaction of the biodiesel production. In order to define a standard, all the samples were diluted to 5 mg/ml. During the dead time due to this process, the samples were sealed and stocked in a freezer at -24°C, this allowed to keep the reaction still and also to reduce the loss of n-Hexane inside the vials due to the evaporation. Before performing the HPLC analysis, all the samples were filtered to reduce the contamination of the column and during the analysis, between each one of them, the column was flushed with the analyte solution to keep it cleaned.
Before loading the sample, some baselines with the spectrum of the column alone were draw in order to determine if some pollutant were present inside it.
Sample's analysis was perform using a High-Performance Liquid Chromatography (HPLC). The HPLC-DAD system was a Shimadzu, C-20AD equipped with a diode array detector Shimadzu, Model SPD-M20A IVDD. The column was a Silicycle *SiliaChrom – AQ 100 C18 (250 x4.6 mm i.d., 5 µm-100* Å*).* The column temperature was kept constant at 40°C, and the mobile phase was composed by Solvent A (Water), Solvent B (Acetonitrile) and Solvent C (2-Propanol/ n-Hexane (5/4 vol/vol)).
The time required for the analysis is approximately 40 minutes:

1. 30% A + 70% B 0 min;
2. 100% B in 10 min;
3. 50% B + 50%C in 20 minutes;
4. Isocratic elution of 50% B + 50% C for at least 10 min.

The injection volume was 20 µl for each sample. The mobile flow entering the column was 1 ml/min and was measured at the exit, by the detector, at 205 nm (Holčapek *et al.*, 1999).
The intervalls of retention time of the common compound that are present before, during and after the reaction are summarize in Table 2.5. This intervalls are related to the method described above, every change to this method will change the retention time of the compounds.

Table 2.5. *Retention time (tR) intervals in minutes of the compounds identified during transesterification reaction (Holčapek et al., 1999).*

Reagents	t_R **[min]**	**Products**	t_R **[min]**
FFAs	1.25-3.10	MGs	3.20-6.00
TGs	20.5-25.00	DGs	16.35-19.60
		FAMEs	9.05-15.25

In order to be sure about the value reported in literature, some standards were used for the retention time determination of triglycerides, FAMEs and free fatty acids. These standards included methyl palmitate (C_{16}^0), methyl stearate (C_{18}^0), methyl oleate (C_{18}^1), methyl linoleate (C_{18}^2) and methyl linolenate (C_{18}^3): the triglycerides present in more percentage inside the rapeseed oil. The standards were purchased from Sigma-Aldrich in stated grades of 99% purity and used as received. The analyses of this standards were useful also to determine the relation between the concentration of the standards and the area resulting in the chromatograms. This relation will be useful during the calculation of the conversion and yield of the reactions.
Furthermore, also a sample containing 5 mg/ml of the initial rapeseed oil was analysed, the resulting chromatogram was useful to estimate the initial concentration of FAs and TGs and their retention times.
Once all the sample were analysed, the conversion of the TGs present inside the oil was calculated using Eq. (2.7). Conversion is in fact defined as the reacted amount of TGs ($C_{TG}^0 - C_{TG}^t$) in respect to the amount of TGs present at time 0 (C_{TG}^0).

$$X_{oil} = \frac{C_{TG}^0 - C_{TG}^t}{C_{TG}^0} \tag{2.7}$$

The mass concentration of the compound can be found through the calibration curves.
Due to a problem related to the HPLC column, the normal C18 was used only during the first part of the analysis. For the part of the monitoring of the reaction was used the same kind of column but instead of the normal phase, it was used the reverse phase AQ C18 Siliachrom. This result in a change of all the retention time of the compounds present inside the reactor. To be sure to recognise the right compound inside the new chromatograms, also the rapeseed oil and the standards were analysed with the new column at different concentrations. With the retention time in the new column, it was possible to determine the change of areas of the FAMEs during the biodiesel production and the conversion of the TGs through the time.

Chapter 3

Results and discussion

The viscosity of the calibration fluid is analysed inside the reactor and inside the off-line standard environment. This analysis defines the calibration curve, useful for the determination of the real viscosity and the different behaviour of the fluid when the environment change. After the calibration curve is defined, the progress of the reaction is determined by the measurement of the viscosity. At the same time, by the analysis of the samples extracted, the conversion and FAMEs production are related to the viscosity trend. This relation is the final task of this thesis: having the graph relating the two variables it is possible to determine the time when the reaction is complete by only measuring of the viscosity.

3.1 Calibration curve

The calibration curves were drawn with the scope of relating the measured (apparent) viscosity of the mixture inside the reactor (online measurement), to the real viscosity measured inside the off-line standard environment(offline measurement). These curves will allow to determine the real viscosity of the samples collected during the real reactions, without the need of measuring them inside the off-line environment. Thus, the only value needed will be the viscosity inside the reactor at the time where the samples were collected.

The calibration points relating the viscosity inside the reactor with the one in the off-line environment are shown in figure 3.1. All the measures displayed were done at the same environmental conditions and at the temperature of 50°C. The relation founded with least square fit is also presented.

In this work, for a matter of time, it was not possible to repeat the experiments and analysis more than one time. All the values analysed by the viscometer have a full-scale range precision of (+/-) 1% of any spindle rotation. This precision is displayed in the first point of Figure 3.1. In all the other points and Figures it will be avoided, in order to simplify the reading.

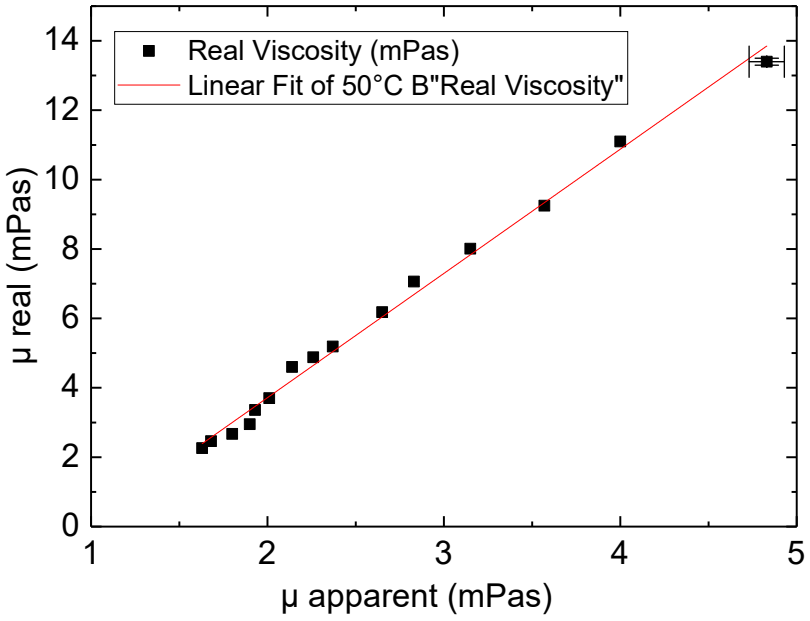


Figure 3.1. *Viscosity relation between inside the reactor and the off-line one; and its linear fitting.*

It was obtained that a linear behaviour: $\mu_{real} = a + b \times \mu_{app}$ can be assumed at different temperatures. In this relation the values of μ_{real} and μ_{app} are the real and the reactor viscosity while a and b are respectively the values of the intercept and the slope of the fitting. Their values at the three different temperatures that were analysed are reported in Table 3.1.

Table 3.1. *Real and apparent viscosity linear fitting at 30, 40, 50°C*

	a	***b***	**Adjusted R^2**
30 °C	-4.07908	3.81624	0.99816
40 °C	-3.74613	3.70008	0.99821
50 °C	-3.44617	3.58093	0.9933

These parameters were fitted with the partial less square method. The determination coefficient R^2 are close to 1 and thus the linear fitting can be considered very reliable. As expected, at different temperature the intercept of the fitting changes, confirming that increasing the temperature, the viscosity of the mixture will decrease. The slopes are only slightly influenced by the temperature and present similar values at three essayed temperatures. Looking at the resulted values, it is possible to say that the relation between the real and the apparent viscosity can be extrapolated to other temperatures and viscosities beyond ones which were analysed. Furthermore, since the slopes has a similar behaviour, the viscosity at different temperatures will change in a similar way that the one analysed. In the case where operational changes are observed as in the agitator, the reactor, the viscometer, or due to a scale up, the measured viscosity will change also. Therefore, considering the real viscosity instead of the apparent one, will be important. In a further step, if a relationship between the reaction yield and conversion are known it will be possible to scale-up this monitoring method to the real plant. We only need the calibration curve relating the two properties in that particular system.

3.2 Viscosity of Oil and Biodiesel

The viscosity inside the reactor changes in a wide range of values. This is due to the temperature change and to the progress of reaction. In order to be sure that the viscosity measured during the reactions was inside the acceptability range, and then that its value was consistent, the velocity of the rotation of the spindle needed to be change. To be able to modify it freely, it is important to determine if the analysed fluids are behaving like a Newtonian one.

To do this experimentally we need to change the shear rate applied to the fluid by changing the velocity of the spindle rotation. Furthermore, to be sure that the fluids had the same behaviour in all the possible temperature, this test was performed while changing the temperature of the fluids. All these measurements where performed inside the off-line standard environment and at controlled temperature as previously described.

In Figure 3.2 the profiles of the viscosity related to the changing of the shear rate applied at the rapeseed oil in a off-line standard environment are shown for isothermal conditions in the range 25°C to 100°C

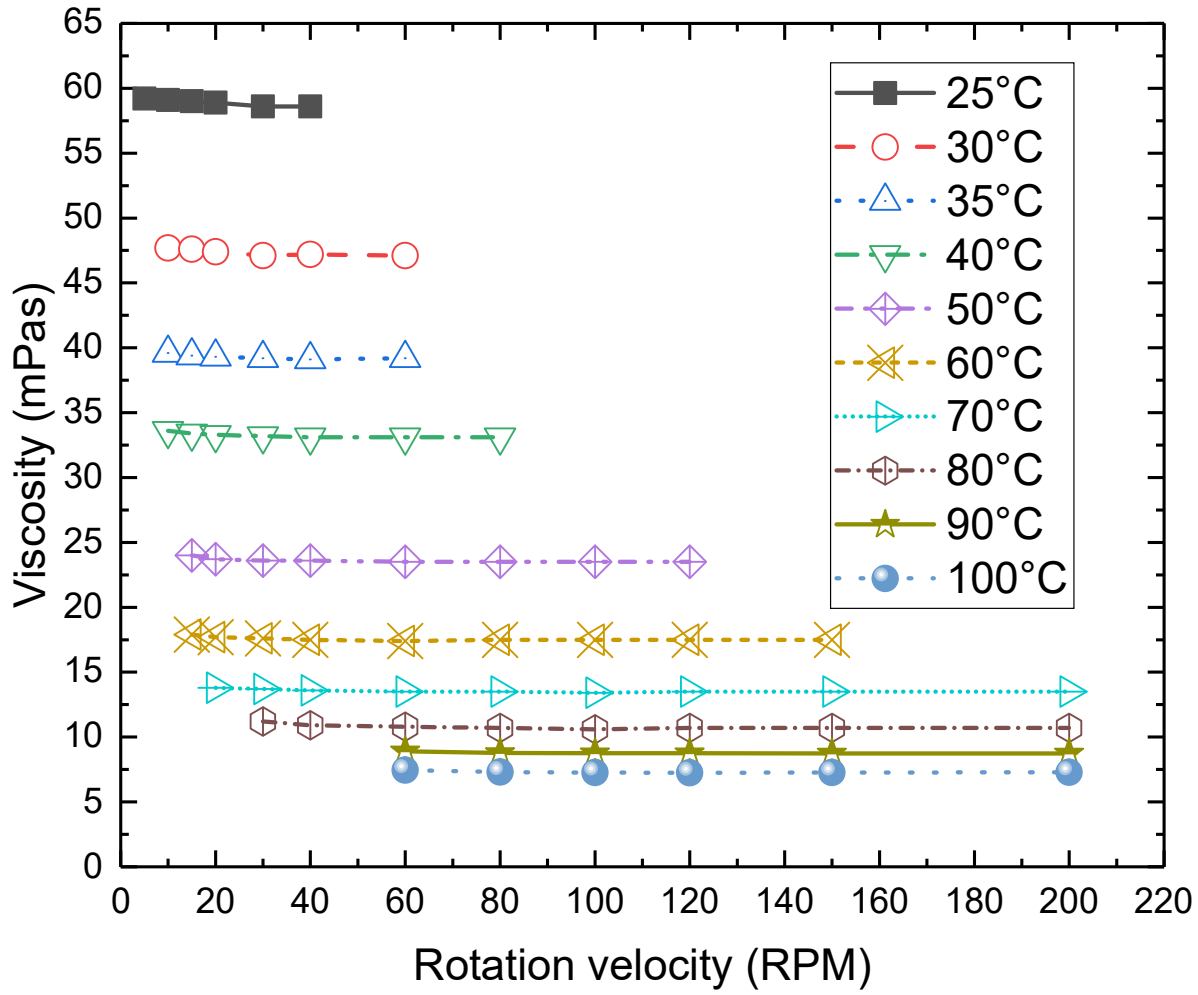


Figure 3.2. *Viscosity changing as function of the shear rate applied at the Rapeseed oil at different temperatures.*

The figure show that at a given temperature, the rapeseed oil under applied shear rate follow almost a Newtonian behaviour, changing its viscosity only by slight values that can be considered within measurement error. A slight increase in viscosity is observed only at the lowest temperatures for very low shear rate. This situation is observed for the measuring range corresponding to the low accuracy of this viscometer. Increasing the velocity of the spindle the measured viscosity stabilizes, confirming that the rapeseed oil can be considered a Newtonian fluid.

In Figure 3.3 the influence of shear rate in the behaviour of the viscosity of Biodiesel are displayed.

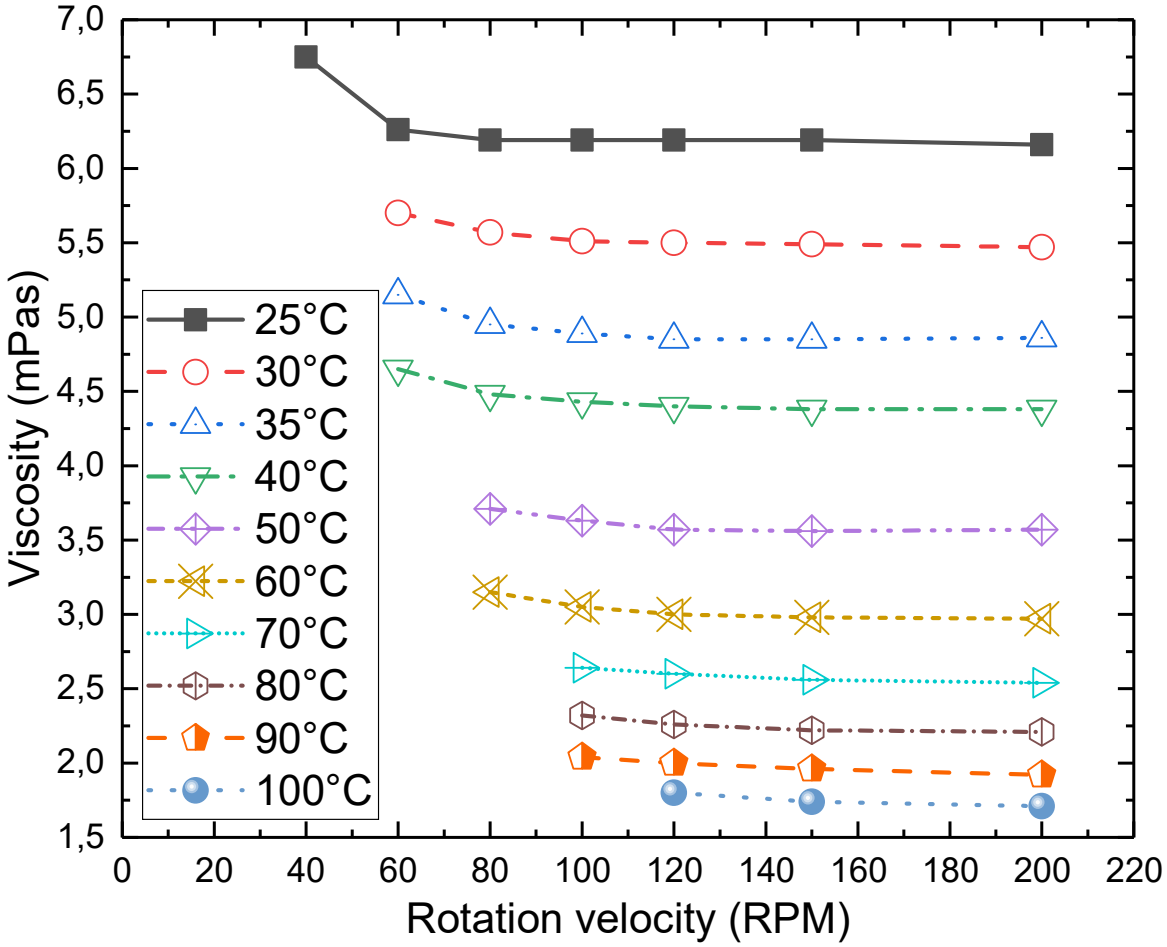


Figure 3.3. *Viscosity changing as function of the shear rate applied at the Biodiesel at different temperatures*

Also in this case it is possible to see that when the applied shear stress is low, there are some slight changes in the viscosity due to the low precision of the viscometer. However, when the shear rate increases, the viscosity of the biodiesel stabilizes at an almost constant value. And, thus, the biodiesel can be considered also as a Newtonian fluid. The fact that all the two fluid, biodiesel and rapeseed oil, can be considered as Newtonian fluids is useful for our work. This means that the analysis of the viscosity of the reaction will not be subject to changes of viscosity due to anomalous rheological behaviour of the fluids, but only because of the variation of the

compound inside the reactor as the transesterification proceeds. Furthermore, this result suggest that it is preferable to monitor the reaction by operating spindle rotation at maximum possible rotational speeds. This will increase the accuracy of the viscometer and the fluid will be analyzed inside the range in which Newtonian behavior can be guaranteed.

3.3 Transesterification reaction monitoring

Once the behaviour of the fluid in different conditions has been determined, it is finally possible to start the monitoring of the transesterification reactions. The first part of the monitoring is the preparation of the reactants. The quantities of the reactants are the ones described in Chapter 2.4: 150ml of oil, 40ml of methanol (molar ratio alcohol/oil r=6) and 1% of catalyst.(Baskar and Aiswarya, 2016) The reactor is then sealed and the viscometer's spindle is placed inside.
Two experiments were performed at 50 °C while the viscosity was measured without the continuous sampling, only to understand if it was possible to perform the monitoring process.
The viscosity of the pure oil and of the final biodiesel produced by the second experiment were analysed both inside the reactor and in the off-line environment of the viscometer. Their values are presented in Table 3.2:

Table *3.2*. *Real and apparent viscosity of pure Rapeseed oil and Biodiesel produced at 50°C*

	Apparent Viscosity (μ_{app}) [mPas]	Real viscosity (μ_{real}) [mPas]
Rapeseed oil	6.64	24.0
Biodiesel mixture	1.84	3.50

Once more the values shown confirm that the measured viscosity inside the reactor and the real one are completely different and the calibration curve is needed.

3.3.1 Viscosity behaviour

While the reaction was taking place, the viscosity inside the reactor was measured by stopping the agitation and turning on the viscometer as previously described. This procedure was performed while registering the time where the viscosity was measured, in order to follow its behaviour through time. The viscosity behaviour of the two experiments is shown in Figures 3.4 and 3.5. As expected, the viscosity decreases fast in the first minutes of reaction and then it remain stable through time until the end of the reaction. All the two experiments behaved in the same way, it can be so assumed that it is the usual behaviour of the transesterification reactions and that the viscosity can be monitored during all the time of reaction.
The relation between the apparent viscosity and the real one is the one found in Chapter 3.1 for the reaction performed at 50°C, and the values are written in Table 3.1. The values of the real viscosity are calculated using the same relation for all the points, so the behaviour of the two curves is exactly the same.

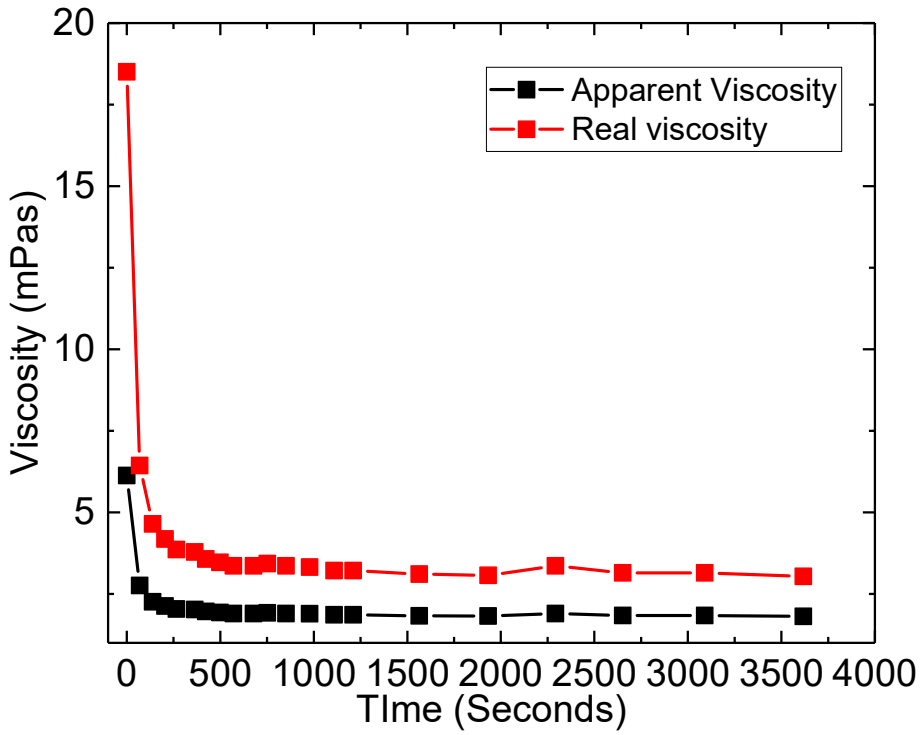


Figure 3.4 *Viscosity behaviour. Operating condition: T=50°C and for 60 minutes, corresponding to the first experiment.*

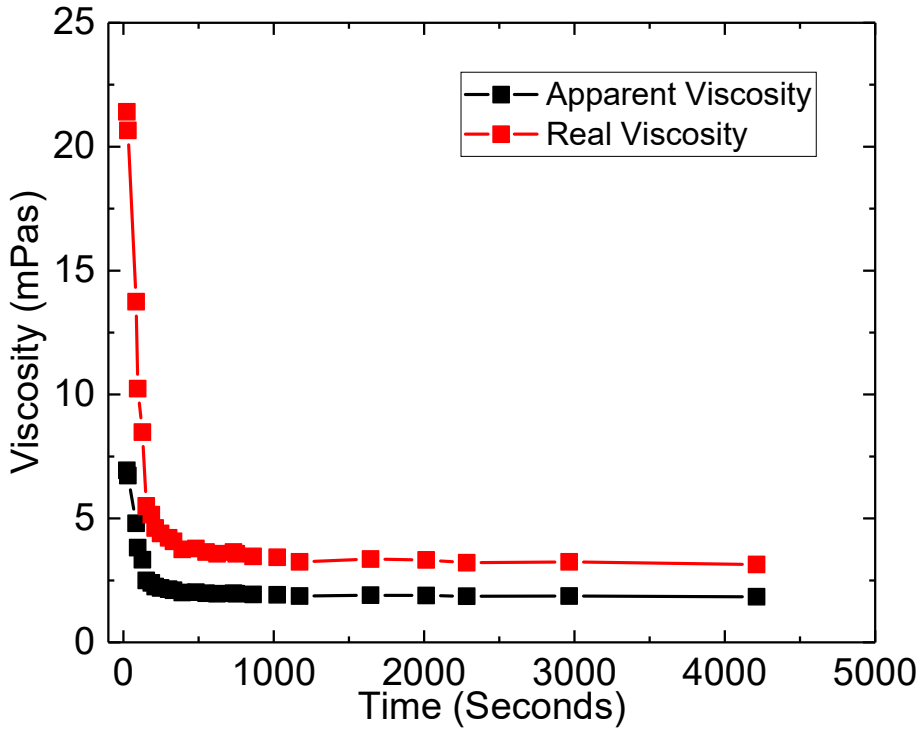


Figure 3.5 *Viscosity behaviour. Operating conditions: T=50°C and 70 minutes corresponding to the second experiment.*

As the velocity of the viscosity decreases fast during the first part of the reaction, waiting just a few seconds before the measurement can change the first value of the viscosity while is measured on the viscometer. Therefore, the first point of the two experiments correspond to different viscosity values. It was in fact impossible to perform the two experiments analysing the viscosity exactly at the same time.

3.3.2 HPLC

The two experiments changed their viscosity values following the described trends. In order to be sure that the variation of viscosity is related to the production of biodiesel, the resulting product has to be analysed with HPLC following the technique described in chapter 2.7.
After performing the reaction, the reactor was drained, and all the products were stocked inside a separating funnel for 2 days in order to separate the various products by their densities. The stratified liquid resulting from this process is shown in Figure 3.6.

Figure 3.6 *Stratified liquids inside the separator funnel.*

When the reaction was successful and the products were the expected ones, in the bottom of the funnel is possible to see the glycerol, together with the unreacted oil and the pollutants present in the feedstock (Figure 3.7). It is possible to see the three different fluids composing it. While in the upper part there should be only valuable products like the FAMEs composing the produced biodiesel, in the bottom the pollutant of the oil and the glycerol are recognisable.

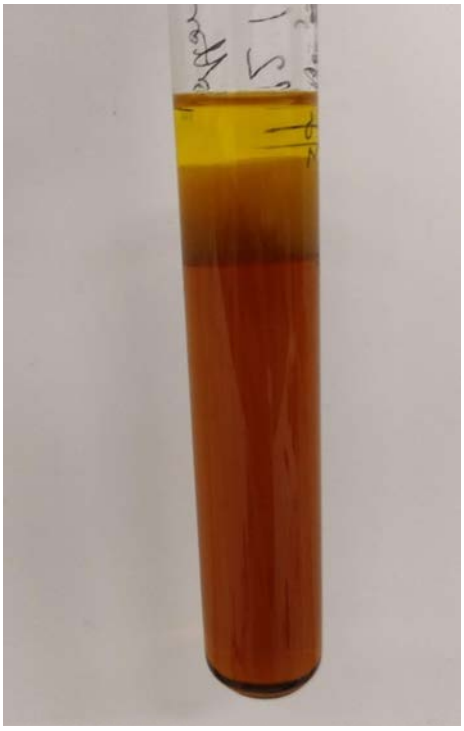


Figure 3.7 *Bottom fluids of the separating funnel.*

The top part of the funnel, the one containing the biodiesel, was then sampled and analysed with HPLC together with the virgin rapeseed oil and the biodiesel produced by the company PRIO. The resulting chromatograms are shown in the next Figures.

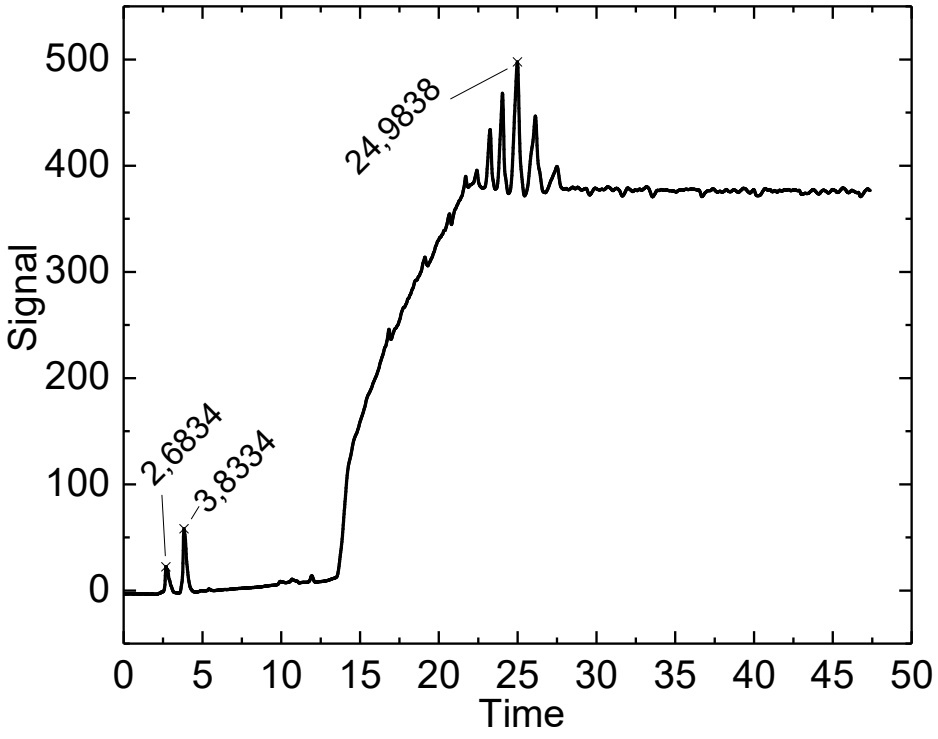


Figure 3.8. *Rapeseed Oil HPLC chromatogram. C18 Column.*

From the chromatogram of the Rapeseed oil which was used to perform the reactions it is possible to identify the peaks that define the fatty acids and the triglycerides present inside the feedstock. It is possible to notice that, as expected, the triglycerides accumulate between 22 and 27 minutes while the fatty acids present inside the feedstock are measured at 2,6 and 3,8 minutes.

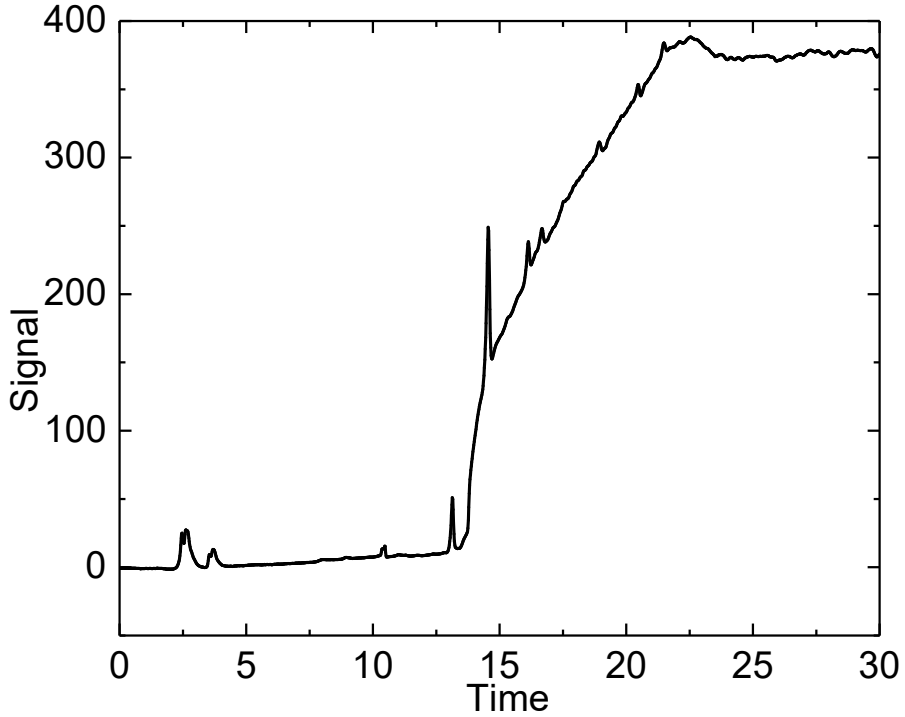


Figure 3.9. *PRIO biodiesel chromatogram. C18 column.*

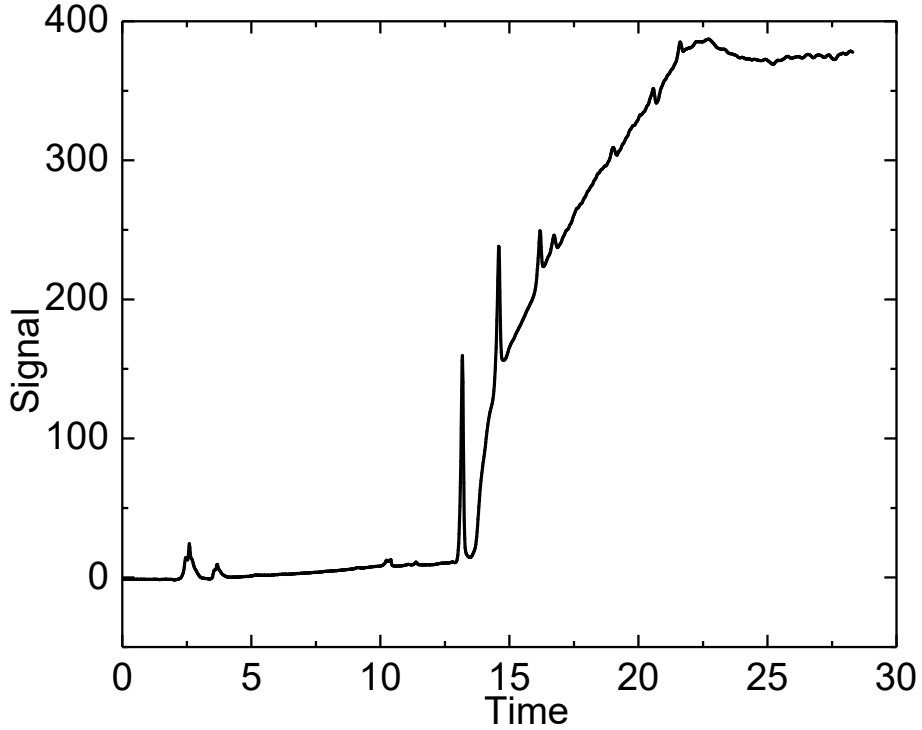


Figure 3.10. *Second experiment biodiesel chromatogram. C18 Column.*

In Figure 3.9 and 3.10 the chromatograms of the biodiesel from PRIO and the biodiesel produced by the second experiment are shown. Comparing the two chromatograms with the one in Figure 3.8 it is possible to notice that the two peaks identifying the fatty acids are almost the same. This means that the fatty acids did not react and are still present in the mixture containing the products of the reaction. The triglycerides peaks tough, disappear almost completely in the biodiesel chromatograms and are substituted by two new peaks at time 13 and 14,5 minutes. These peaks are the one that identify the FAMEs produced and confirm that the product of our experiments is in fact biodiesel. The different areas of the peaks and their relative percentage of the three chromatograms are shown in Table 3.3, 3.4 and 3.5. The values displayed on the table confirm the assumptions: the triglycerides present in the rapeseed oil decrease in the biodiesel and the peaks that represent the FAMEs, two at 13 and 14.5 minutes and other two around 16 minutes are present in all the two biodiesels: the one from the second experiment and the PRIO's. The value of the areas of the peaks between the PRIO biodiesel and the one produced by the second experiment is not comparable in a quantitative way. The biodiesel from PRIO was, produced using rapeseed oil, but it was produced 6 months before with a different feed of rapeseed oil. The percentage of different type of FAMEs inside the biodiesel could not be the same, also if the feedstock is of the same kind. Therefore, the peak at 13 minutes is bigger in our product than the one in PRIO's. The percentage of FAMEs between the biodiesels are different and can vary from different supplier.

Table 3.3. *Rapeseed oil areas of peaks with retention times and relative percentage.*

Retention time [min]	Area	Height	%Area
2.7	7.08217	24.1667	1.0678
3.833	16.6804	60.3035	2.515
16.834	117.884	18.2795	17.7742
19.117	55.9231	47.1998	8.4319
20.334	51.1318	55.3553	7.7095
20.684	25.3319	65.7733	3.8195
21.7	75.6103	86.7639	11.4002
22.417	65.9299	82.3898	9.9407
23.25	59.5855	109.087	8.9841
24.034	59.9627	132.153	9.0409
24.944	71.1666	148.25	10.7302
29.117	45.6977	81.046	6.8901
27.501	11.248	22.5226	1.6959

Table 3.4. *PRIO biodiesel areas of peaks with retention times and relative percentage.*

Retention time [min]	Area	Height	%Area
2.467	3.68924	26.0612	3.9974
2.617	8.51627	28.3041	9.2276
13.133	4.56402	38.7747	4.9452
14.55	35.0539	116.158	37.9817
16.133	7.91966	27.5118	8.5811
16.667	5.00455	15.2481	5.4225
18.933	15.499	13.036	16.7935
19.817	4.48009	6.8828	4.8543
20.467	4.5661	11.2294	4.9475
21.483	2.99869	9.96546	3.2492

Table 3.5. *Second experiment areas of peaks with retention times and relative percentage.*

Retention time [min]	***Area***	***Height***	***%Area***
2.59	1.46136	13.3112	1.6524
2.617	0	0	0
13.184	18.2592	146.974	20.6468
14.584	31.1514	104.601	35.2248
16.184	8.17135	39.1476	9.2398
16.734	5.0566	14.233	5.7179
19.017	11.8955	9.87295	13.4509
20.584	8.17666	13.325	9.2459
21.617	4.26394	11.058	4.8215

3.4 Viscosity and reaction progress correlation

This analysis had the scope to determine the quantity of FAMEs present inside the reactor, in order to trace its change. After the monitoring of the viscosity inside the reactor, it is necessary to understand if the change of viscosity is in effect related to the production of biodiesel. In order to do so, other two experiments were performed, one with molar ratio r=4 at 40°C and the other one with r=6 at 50°C. In all the two conditions the resulting viscosity change had the same behaviour this means that, changing the conditions and so the concentration inside the reactor, the viscosity follow always the same trend. However, in these experiments, each time that the viscosity was measured, also a sample was taken from the reactor. The sample was then immediately quenched and stored at -24°C. All the samples, each one related to a viscosity measure and so value, were then analyse with the HPLC. Furthermore, looking at the TGs areas, it was possible to calculate a hypothetic conversion with Eqn. (2.8), using the fitting parameters of the curve related to the standards of rapeseed oil.

3.4.1 Rapeseed Oil standard

As explained before, for this part of the analysis it was used a C18 reverse phase column instead of the normal one. This results in a different retention time for each compound than the one presented in literature. It was then necessary to repeat the analysis of all the standards of the FAMEs and TGs in order to have the new retention times.

The chromatogram of the rapeseed oil at defined concentration is shown in Figure 3.11. In this chromatogram it is shown the rapeseed oil analysed with the reverse phase column. Inside it is possible to locate the different peaks that define the compounds that are present inside the rapeseed oil naturally before the performing of the experiment: the TGs and the fatty acids. The TGs has three different retention times in the chromatogram: at 4.2 min, 23.5 min and 25 min. These are the TGs peaks to which change will have to be follow during the experiments in order to calculate the TGs consumption and conversion.

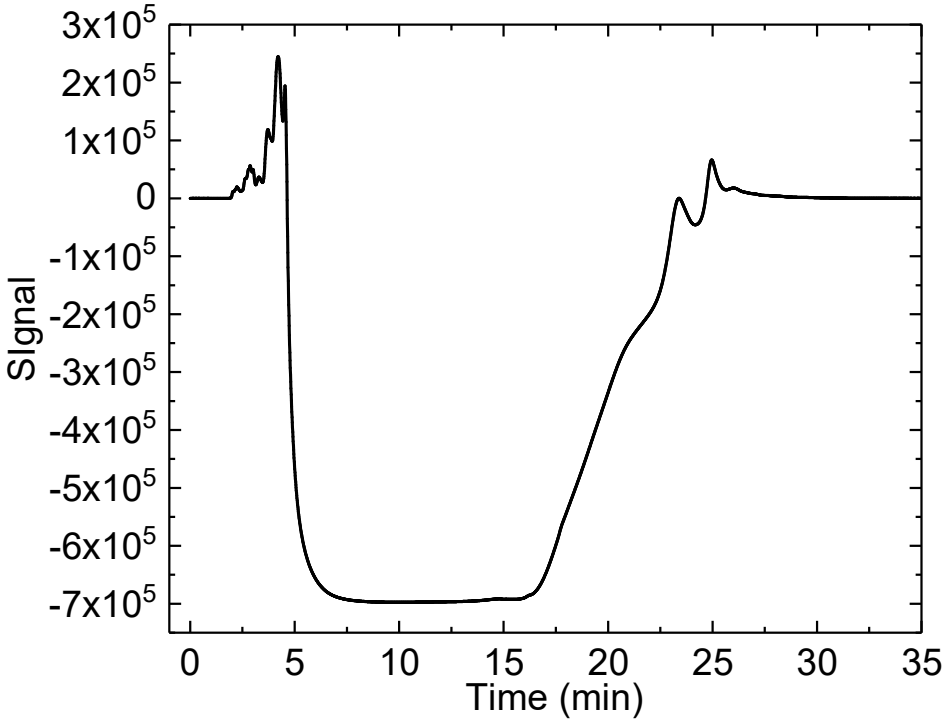


Figure 3.11. *Rapeseed oil (5mg/ml) HPLC chromatogram.*

The fatty acids, in this chromatogram can be identify within the first two peaks that are present around 3,28 min and 3,71 min. These two peaks are still present in the chromatogram of the reacting solutions and get overwritten by the FAMEs peaks. The areas of the peaks with the correspondent retention time are show in Table 3.6. This table is useful to determine the main peaks in the chromatogram and compare them within the standards.

Table 3.6. *Rapeseed oil's peaks areas and retention times.*

Retention time [min]	***Area***	***%Area***
2.05	18019	0.22884
2.22	54083	0.68686
2.86	28316	0.35962
3.00	20370	0.2587
3.28	112888	1.4337
3.71	774644	9.83813
4.20	2062488	26.194
23.40	2554498	32.4426
24.95	2248585	28.5575

3.4.2 Standard fitting

The rapeseed oil and the FAMEs, that present in the biodiesel produced from the oil, were used as standards for the peak's areas and retention times determination. For each of them, 4 samples, at known and different concentrations were prepared in order to find the correlation between the concentrations and the areas of the peaks. In the Rapeseed oil case, the analysed concentrations are: 2 mg/ml, 3mg/ml and two samples at 5 mg/ml, to be sure that a good result can be achieved. In the case of the FAMEs standards, the concentration analysed to determine

the calibration curve were: 1 mg/ml, 2 mg/ml, 5 mg/ml and 10 mg/ml for each of them. For each standard, the relation between the concentration of the sample and the areas of their peaks are represented in the fitted line in Figure 3.11.

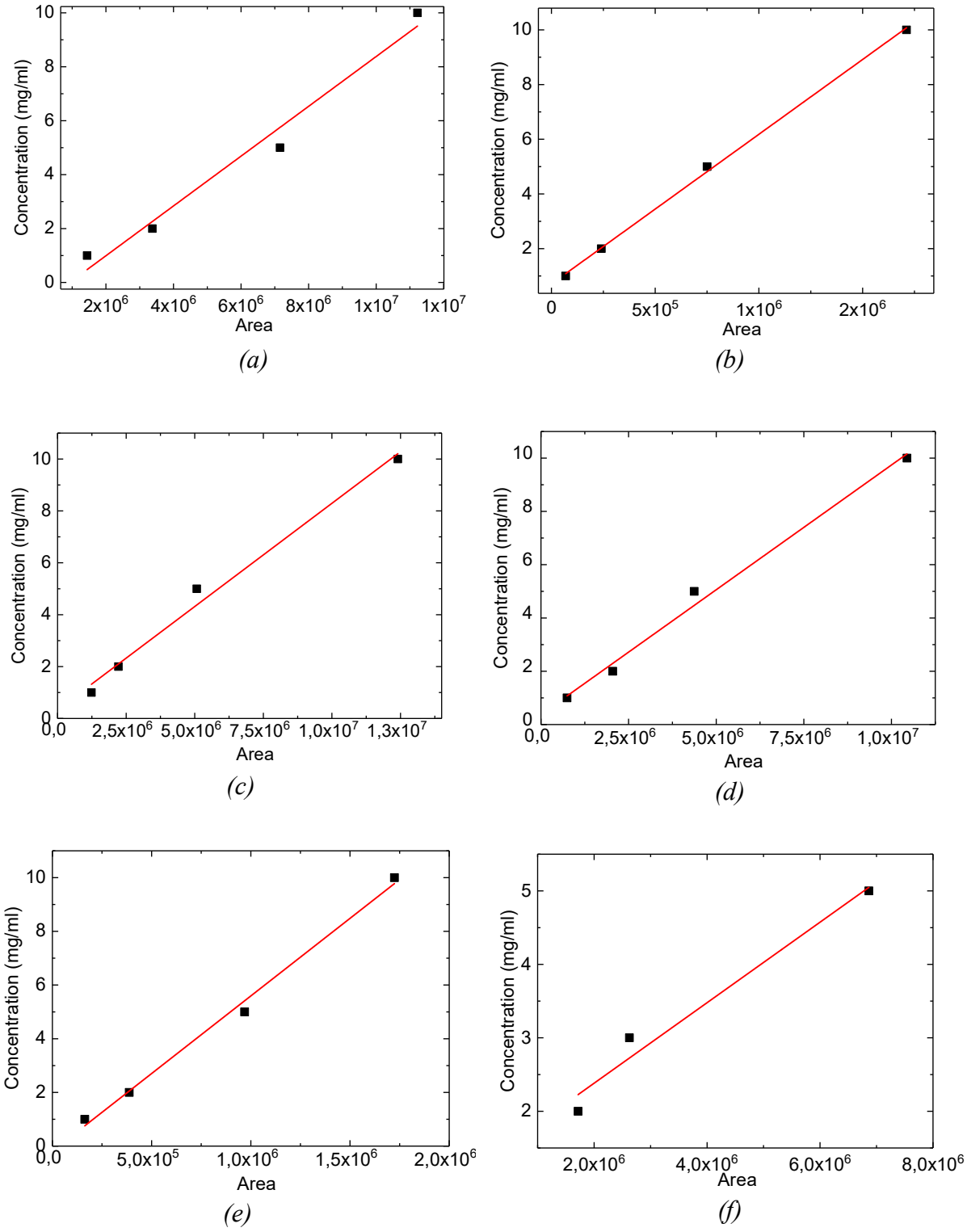


Figure 3.11. *Concentration and Areas of the various fatty acid methyl esters (**a.** Oleate, **b.** Stearate, **c.** Linolenate, **d.** Linoleate, **e**. Palmitate) and **f.** rapeseed oil.*

All the standards were fitted linearly. The values of the intercepts, slopes, adjusted R^2 and retention times found from the HPLC analysis of the FAMEs are presented in Table 3.7.

Table 3.7. *Standard's fitting parameters and retention times.*

	Intercept	Slope	Adj. R^2	Retention time
Linoleate	0.37663	9.36581E-07	0.98786	2.10
Linolenate	0.33581	7.9576E-07	0.98324	2.06
Oleate	-0.85525	9.235E-07	0.965	2.70
Palmitate	-0.18756	5.78081E-07	0.99137	2.80
Stearate	0.71151	5.4667E-06	0.99854	3.00
Rapeseed Oil	1.32383	5.30989E-07	0.969	-

In the case of the rapeseed oil the retention time of the TGs are more than one and were already displayed in section 3.4.1. To determine its fitting parameters, the area used to relate the peaks with the concentration is the sum of all the areas of the peaks that were related to the TGs. This means that in the chromatogram of the samples during the reaction, in order to find the concentration of the TGs, it will be necessary to use a sum of the areas, and not the one of each peak.

3.4.3 Biodiesel Standard

A sample of the PRIO biodiesel was analysed by the same reverse column as well. This solution was adopted to assure that the product of the reaction was similar to that one produced by PRIO and that no contaminants were present. The chromatogram resulting from this sample is shown in Figure 3.12. In this Figure is possible to notice that there are no peaks present in the latter part of the chromatograms(20-22 minutes), but they are all present in the first part, between 2.0 and 3.0 minutes. This samples of biodiesel were taken directly from the plant of production, but it was taken after all the process, it means that it was already washed, and treated in order to remove all the reactants, the glycerol and catalyst oddments. This is the difference between the sample taken directly inside our reactor and the one from PRIO. In the ones coming from our reactor will be present some compounds that here are not, and they will show themselves through their peaks in the chromatograms.

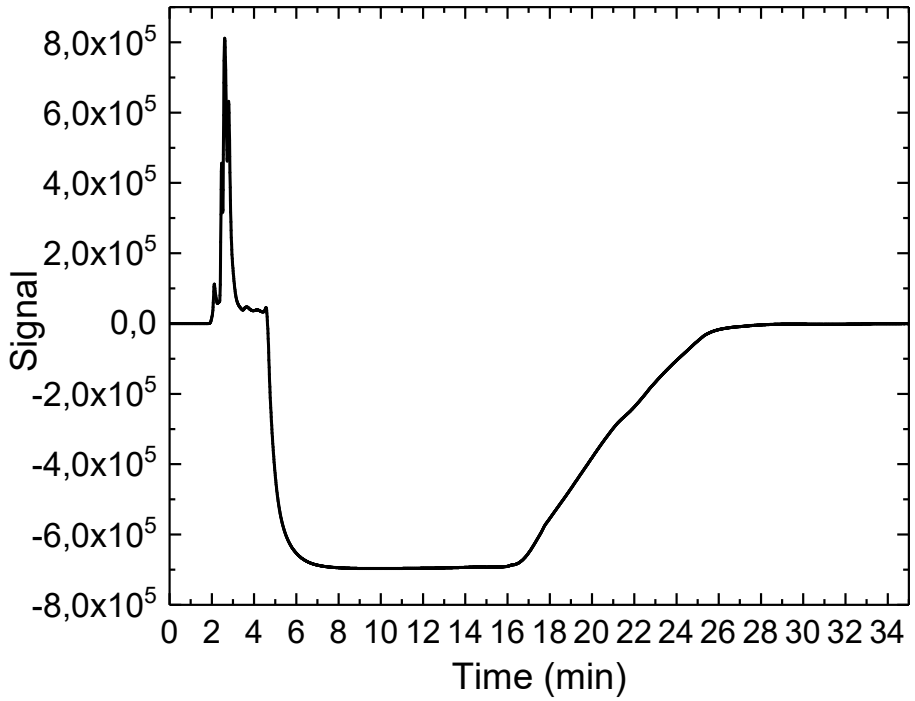


Figure 3.12. *PRIO biodiesel chromatogram*

The retention times, areas and relative percentage of the relevant peaks present in this chromatogram are reported in Table 3.8.

Table 3.8. *Retention times, area and percentage for the peaks present in PRIO biodiesel*

Retention time [min]	***Area***	***%Area***
2.11	567697	12.13
2.46	426708	9.11
2.61	2650724	56.63
2.79	1035496	22.12

All the values of the retention times of the peaks are closer to the one of the standards ones reported in Table 3.7. This is another confirmation that the FAMEs are determinable by this method and they will be present in the first 4 minutes of the chromatogram. Furthermore, this biodiesel was produced by the same feedstock but in a different time of the year. The difference between rapeseed oil used to produce the two different biodiesels will result in similar composition but not identical. This will bring to a difference between the retention time of the peaks present in the chromatograms, which will be delayed by some seconds one to each other's. The retention time between the two can in fact be slightly different (some seconds).

3.4.4 Reaction and monitoring results

The reaction described in this section was performed with a molar ratio of r=6 and at 50°C: the best condition in order to have a good conversion of the TGs. As explained before, the samples were taken simultaneously within the viscosity measurement in order to find a correlation between the production and consumption of the products and reactants. The chromatogram resulting from the analysis of the first and last samples are shown in the next figures.

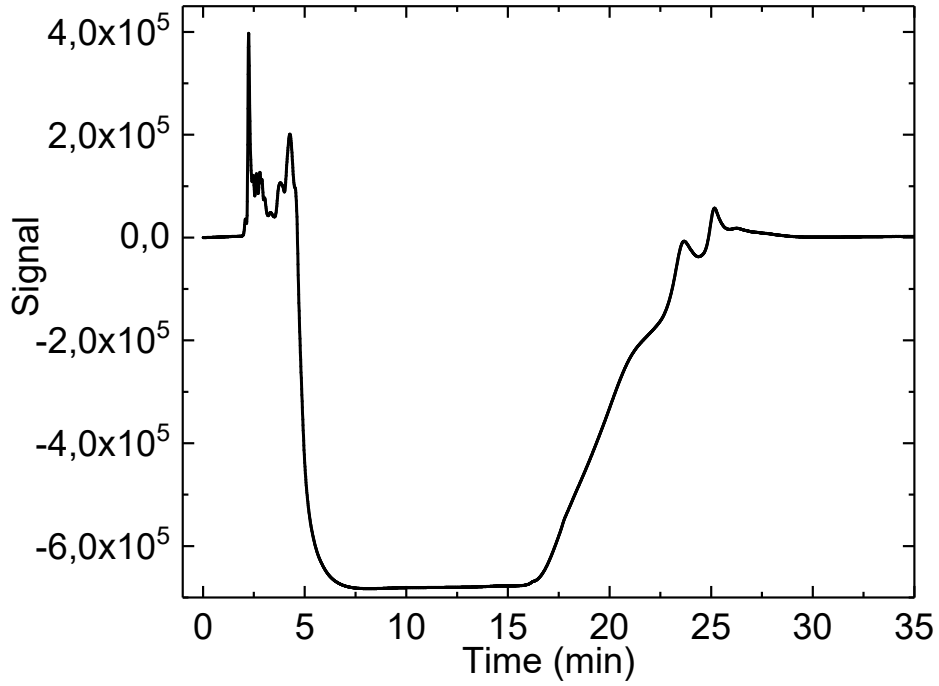
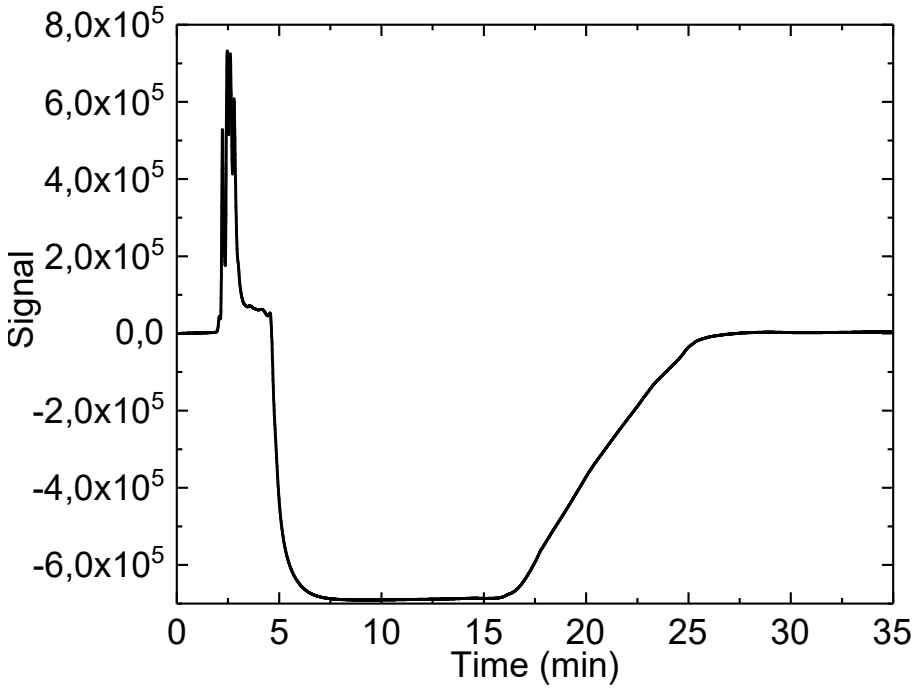
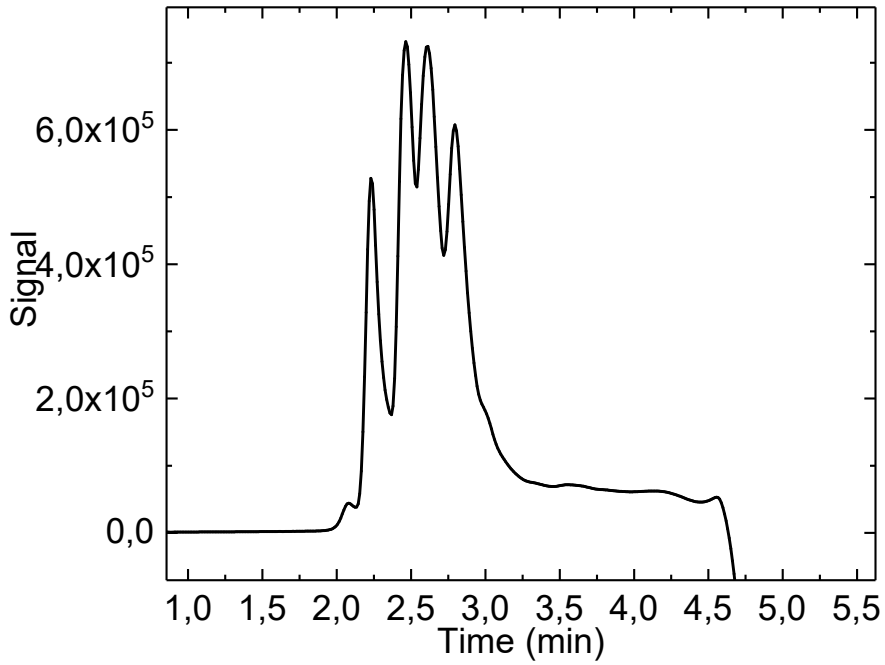


Figure 3.13. *Chromatogram of the first sample taken at t=25seconds.*

Looking at the Figure 3.13 and 3.14.a it is possible to notice a change in the peaks detectable. The first thing that comes to the eyes is that the two peaks determining the TGs located at times 25, almost completely disappear in the last chromatogram. This means that the TGs that were present in the oil were consumed and FAMEs were produced. The latter tend to accumulate in the first part of the chromatogram, as evidenced in Figure 3.14.b. Furthermore, in the first part are present some peaks that in the first chromatogram are detected around 4.5 minutes, but in the last one are all accumulated between 2.0 and 3.5 minutes. These times correspond to the times were, also in the standards, the FAMEs were detected. This let us understand that these peaks are the one related to the FAMEs and that they are present in our sample as products of the reactions.



(a)



(b)

Figure 3.14. ***(a)*** *Chromatogram of the last sample at t=70 minutes.* ***(b)*** *Focus on the first part of the chromatogram (1 to 5 minutes) detecting the FAMEs and the glycerol produced.*

The values of the retention times, areas and relative percentage of the peaks founded in the first and last samples are reported in the following tables. These two samples can describe the changing of the compounds from the beginning of the reaction up to the end. The other chromatograms and Tables reporting the value of all the analysed samples are shown in the Appendix B.

Table 3.9. *First sample: Areas and retention time of peaks with their determination.*

	Retention time(min)	Peak time (min)	Area	% Area
Linoleate	2.10	2.08	72201	0.81517
Glycerol	-	2.24	2123575	23.9757
Linolenate	2.06	2.46	87927	0.99272
Oleate	2.70	2.61	178293	2.01298
Palmitate	2.80	2.79	139221	1.57184
Stearate	2.90	2.89	40463	0.45684
		3.13	55587	0.62759
		3.8	637719	7.20002
TG	-	4.26	1722744	19.4502
TG	-	23.66	1976769	22.3183
TG	-	25.16	1822684	20.5786

Table 3.10. *Last sample: Areas and retention time of peaks with their determination.*

	Retention time(min)	Peak time (min)	Area	% Area
Linoleate	2.10	2.08	136137	1.54872
Glycerol	-	2.24	3730884	42.4433
Linolenate	2.06	2.46	1980554	22.5312
Oleate	2.70	2.61	1337428	15.2149
Palmitate	2.80	2.79	1605268	18.2619
Stearate	2.90	2.89	0	0
		3.13	0	0
		3.8	0	0
TG	-	4.26	0	0
TG	-	23.66	0	0
TG	-	25.16	0	0

Comparing the peak time within the standards it is possible to relate the peaks to the compound that generate them. In Table 3.9 and 3.10 the peaks relating TGs and FAMEs can be easily identified. Their retention times are quite similar to the ones of the standards, confirming the fact that they are the compound that were expected.

The peak at time 2.24 min is present in all the samples taken directly from the reactor, while it is not present in the biodiesel coming from PRIO (Figure 3.12). This peak can be related to the glycerol, which is present during the reaction while it is not present in PRIO biodiesel because the latter was already purified from by-products. Being one of the products of the reaction it increases in time and, because of the stoichiometry, it becomes one of the biggest peaks in the chromatograms of our samples.

In order to follow the conversion of the TGs through time, the three peaks related to them were summed together and analysed as they were the same compound. This allow to follow the trend of their consumption in relation to the viscosity changes. The graph showing the viscosity trend and the consumption of the TGs in all the samples taken is shown in Figure 3.15.

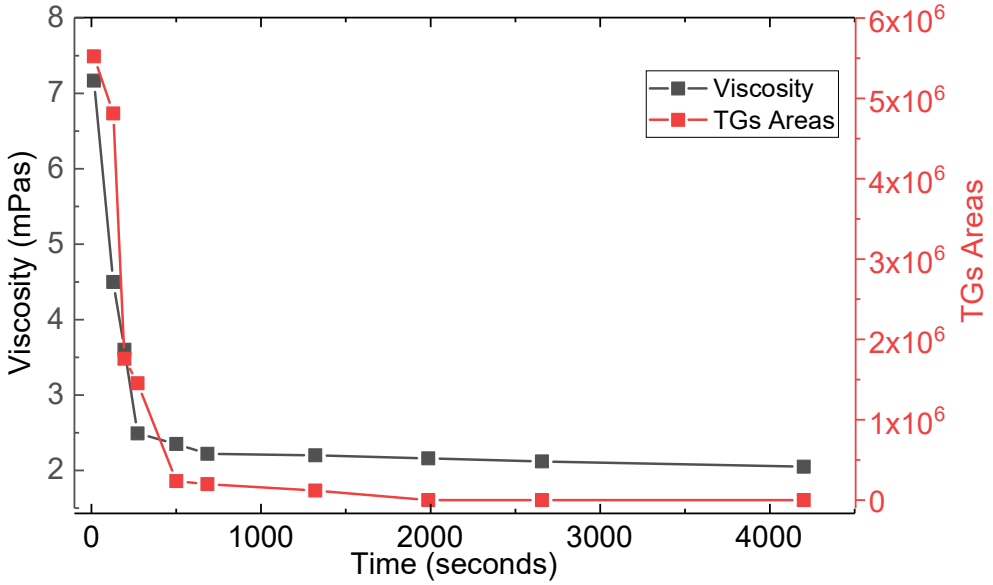


Figure 3.15. *Viscosity and TGs area changes with time of reaction.*

Looking at the viscosity behaviour (black curve) it can be concluded that also in this experiment, like the one already seen in chapter 3.3.2, the viscosity decreases faster in the first five minutes, keep decreasing till minute 22. Then it stays almost stable until the end of the measurements. Comparing the viscosity changes in time with the corresponding changes on the sum of TGs areas, it is notable a similar behaviour. The TGs consumption follow almost the same trend of the viscosity and this means that the viscosity can be an indicator of their consumption.

If we look instead at the conversion of the TGs the trend is reverse of the one of TGs areas, i.e. the conversion increase while the viscosity decreases as shown in Figure 3.16.

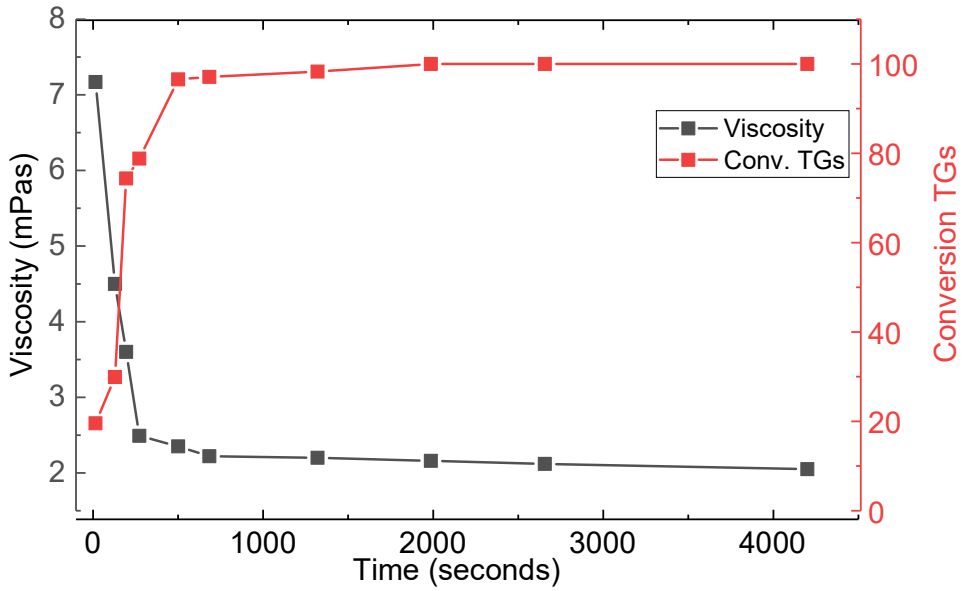


Figure 3.16. *Viscosity and TGs conversion through time.*

In this graph the reaction seems to reach 100% of conversion. This conversion is related to the concentration of the TGs through the oil standards. It is only an approximated conversion because it is based on the fact that the peaks are almost completely disappeared in the chromatograms. It could also be that some traces of the TGs are still present, but their peaks are not relivable. It can be notices that the maximum conversion it is reached at the same point as in Figure 3.15 (around 22 minutes).

To be sure that the reaction progress is producing FAMEs and glycerol, also the study of the product is important. In Figure 3.17 the graphic relating the FAMEs production (sum of areas) related to the same viscosity is displayed.

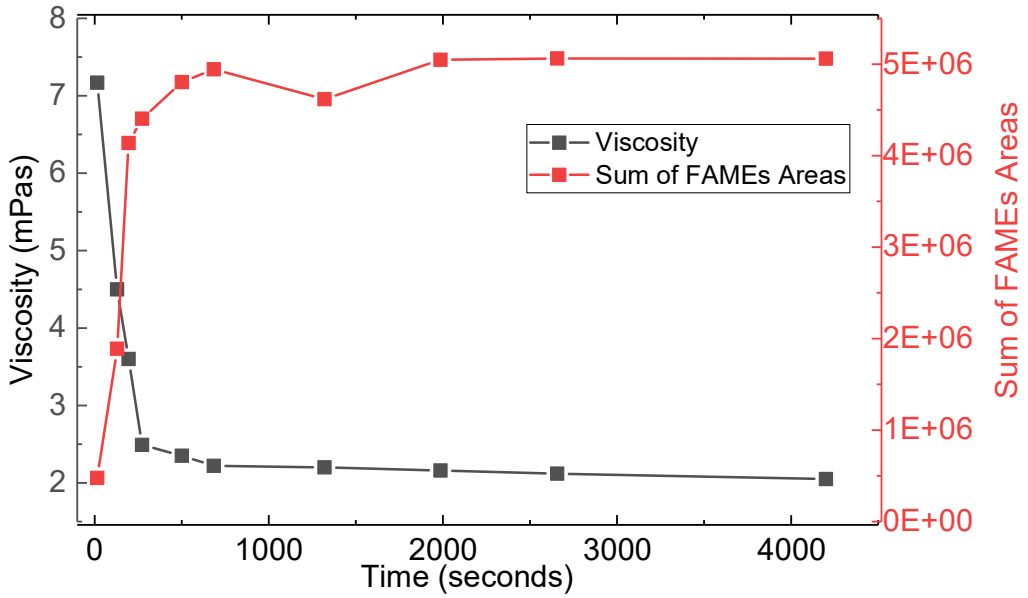


Figure 3.17. *Viscosity and FAMEs area through time.*

Looking at the production of FAMEs, the trend followed is almost the same as before. It is, in fact, possible to follow the trend of the FAMEs production and relating it to the trend of the viscosity. As in Figure 3.15 and 3.16 for the TGs, the FAMEs line reaches an almost stable value at 22 minutes, together with the viscosity. After the 22 minutes the areas of the FAMEs peaks are practically the same (the difference is very low and can be neglected) and also the viscosity measured change slightly between the last four points, as displayed in the figure. This little change can be reconduct to the change of the DG and MG formed by the reactions to the final FAMEs but also to the loss of some of the methanol from the reactor to the environment.

The values of all the point displayed in the previous figures are resumed in Table 3.11. In this table the value of the real viscosity of the fluids, found using the calibration curve of paragraph 3.1, are also present.

Table 3.11. *Value of viscosity, Areas of FAMEs, Areas of TGs and conversion with time of reaction.*

Time (seconds)	Viscosity (mPas)	Real viscosity (mPas)	FAMEs Areas	TGs Areas	Conv. TGs
15	7.170	22.230	477642	5522197	19.6 %
129	4.500	12.668	1889044	4810889	29.9 %
195	3.600	9.445	4137795	1759463	74.4 %
273	2.490	5.470	4403967	1454557	78.8 %
501	2.350	4.969	4804010	236943	96.5 %
684	2.220	4.504	4942937	199183	97.1 %
1320	2.200	4.432	4616538	118764	98.3 %
1986	2.160	4.289	5047438	0	100 %
2657	2.120	4.146	5062026	0	100 %
4200	2.050	3.895	5059387	0	100 %

3.4.5 Reaction at r=4 and T=40°C

In order to understand if the viscosity can be used as a monitoring parameter, it is necessary to understand if its behaviour change when there are changes in the reaction's parameters.
Using rapeseed oil, methanol and sodium methoxide, the best ratio to use is 6 and the temperature between 50 and 60°C. To be sure that the reaction can be monitored in different conditions, in this second experiment it was used a molar ratio of 4 and the reactor heated only at 40°C. These are the worst condition that can be used while keep allowing the forwarding of the reaction to biodiesel production. Analysing the best and the worst reaction, it is possible to determine if the monitoring of the viscosity can be suited to different type of transesterification with different reactant and reacting conditions.

In Tables 3.12 and 3.13 the retention times, area and relative percentage of all the peaks present in Figure 3.18 and 3.19 are presented. These tables help to understand the variation through the time of the mixture sampled form the reactor. Following the retention time of the standards in chapter 3.4.2 and the results of the reaction analysed in the previous chapter, it was possible to identify all the peaks presents in the chromatograms.

Table 3.12. *Second sample: Areas and retention time of peaks with their determination*

	Retention time(min)	Peak time (min)	Area	% Area
Linoleate	2.10	2.08	287175	1.59696
Glycerol	-	2.24	1.4E+07	76.1474
Linolenate	2.06	2.47	0	0
Oleate	2.70	2.62	0	0
Palmitate	2.80	2.8	0	0
Stearate	2.90	3.29	114584	0.63719
		3.83	84256	0.46854
TG	-	4.39	969079	5.38897
TG	-	24.03	1753005	9.74832
TG	-	25.4	1081230	6.01263

Table 3.13. *Last sample: Areas and retention time of peaks with their determination*

	Retention time(min)	Peak time (min)	Area	% Area
Linoleate	2.10	2.08	32387	0.5394
Glycerol	-	2.24	911959	15.1887
Linolenate	2.06	2.47	2188223	36.4448
Oleate	2.70	2.62	1422693	23.6949
Palmitate	2.80	2.8	1427810	23.7801
Stearate	2.90	3.29	0	0
		3.83	0	0
		4.2	21139	0.35207
TG	-	4.39	0	0
TG	-	24.03	0	0
TG	-	25.4	0	0

The second and last samples chromatograms are shown in Figures 3.18 and 3.19.

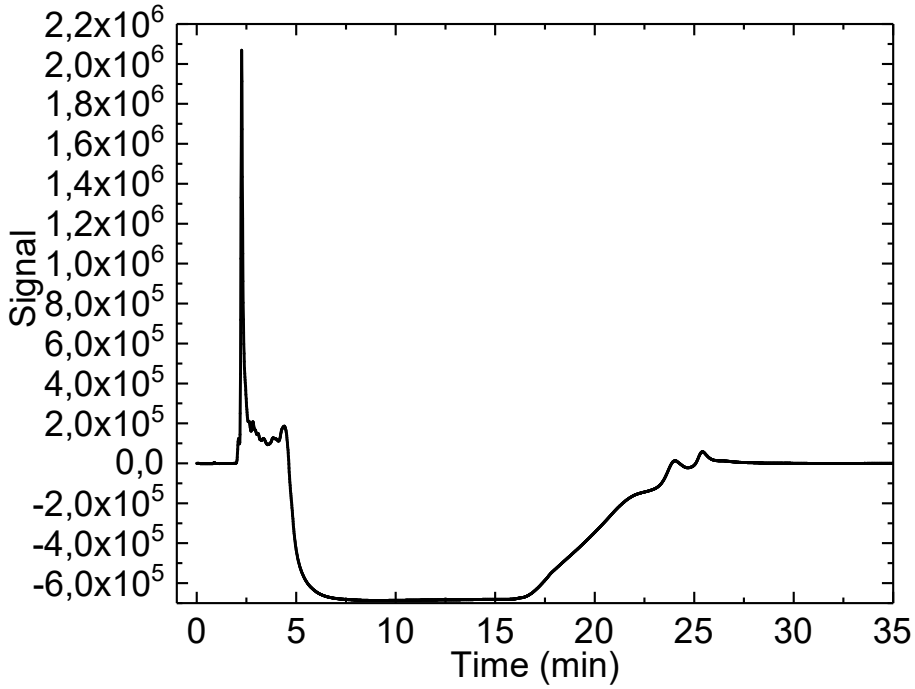
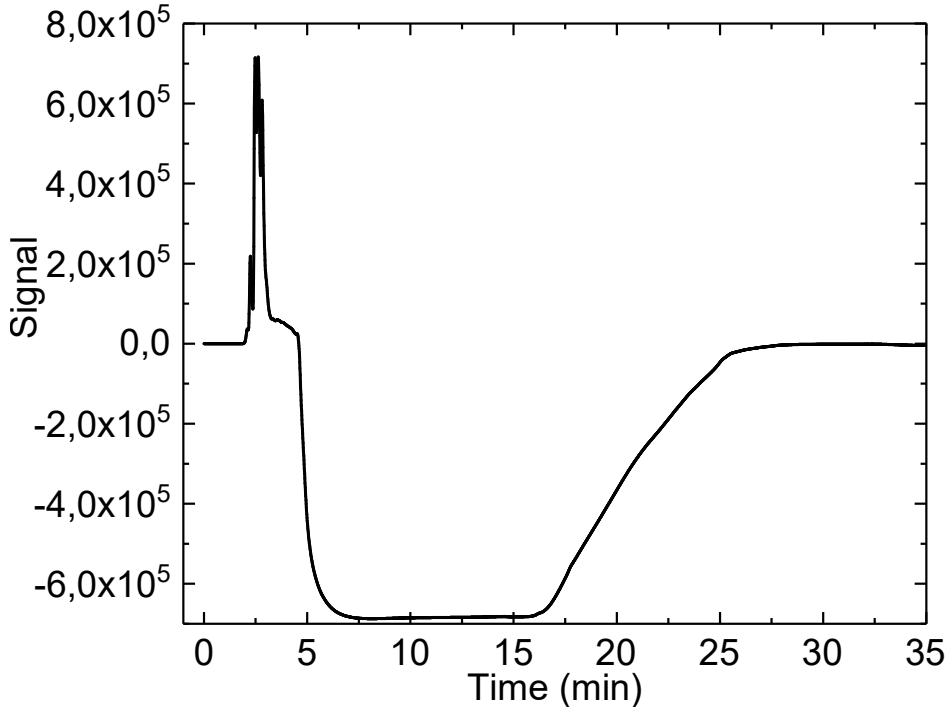
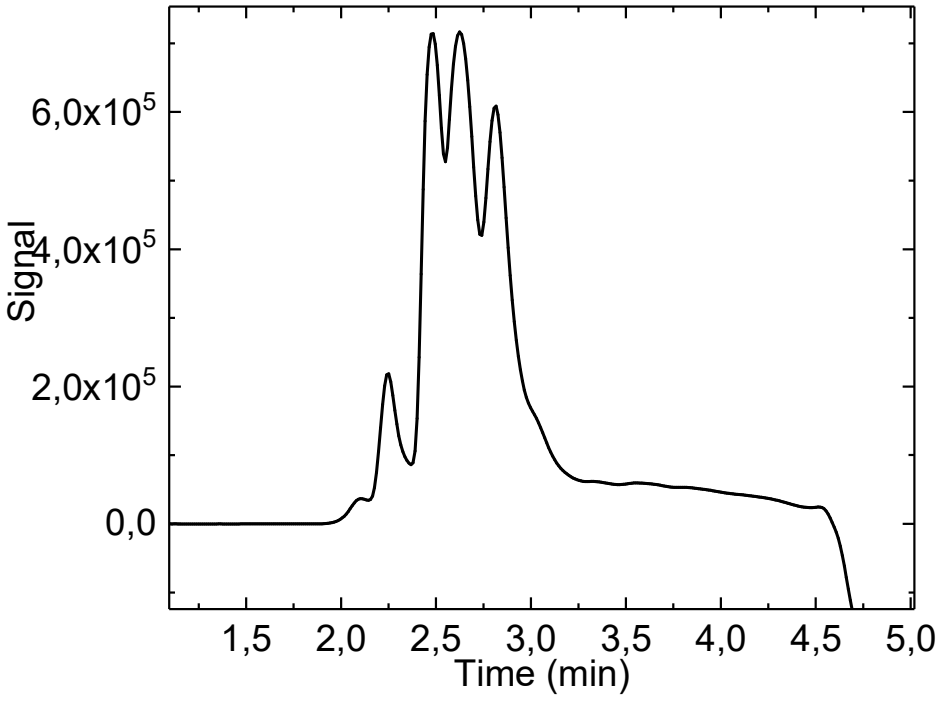


Figure 3.18. *Chromatogram of the sample taken at t= 2.08 minutes.*



(a)



(b)

Figure 3.19. ***(a)*** *Chromatogram of the last sample at t=70 minutes.* ***(b)*** *Focus on the first part of the chromatogram (1 to 5 minutes) detecting the FAMEs and the glycerol produced.*

In Figure 3.18 the peaks referring to the glycerol are very high. This was probably due to some problem within the analysis. The information that can be derived from these two figures is the consumption of the TGs between the first one and the last one. The peaks that were related to the TGs (two at 25 minutes and one at 4.2), while being present in the first chromatogram, result completely disappeared in the last one. Furthermore, the FAMEs peaks seem to increase through time and reveal themselves at almost the same time of the reaction previously analysed (at r=6 and T=50°). Figure 3.19.b shows a focus in the first part of the chromatogram, where the FAMEs peaks accumulates.

Looking at the chromatograms and at the tables, it is possible to notice that the reaction behaves quite similarly to the ones described in the previous section. The TGs appears only in the first samples and completely disappears in the last ones while the FAMEs peaks don't appear in the firsts but increase consistently in the last ones. In order to confirm our theories, it is necessary to analyse the behaviour of the TGs and FAMEs in relation to the viscosity changes. In Figures 3.20, 3.21 and 3.22 the same three relations analysed in chapter 3.4.4 are shown.

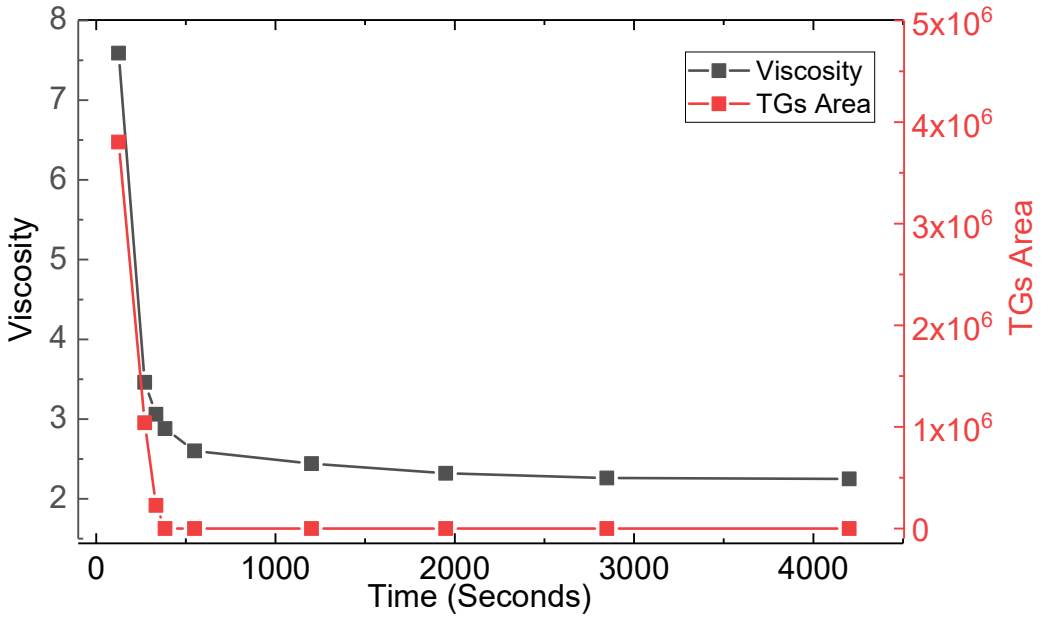


Figure 3.20. *Viscosity and TGs area changes with time.*

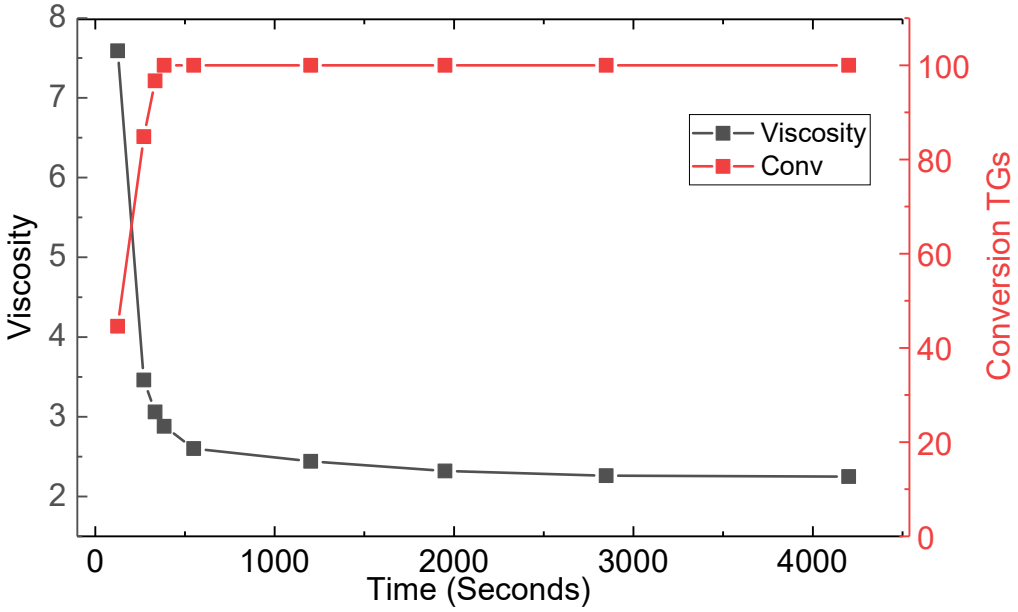


Figure 3.21. *Viscosity and TGs conversion change through time.*

It can be observed that the behaviour of these two graphs is generally quite similar to the ones shown in the previous chapter. The TGs areas and conversion behave in the same way as in the previous case. In this case tough, the maximum of the conversion is reached in less time. However, the viscosity after that keeps changing until it reaches a stable value. This means that all the TGs were consumed and converted in some products that could be DGs, MGs or FAMEs and thus, the viscosity keeps changing after the ending of the TGs: the second and third reaction of Figure 1.9 are still ongoing.

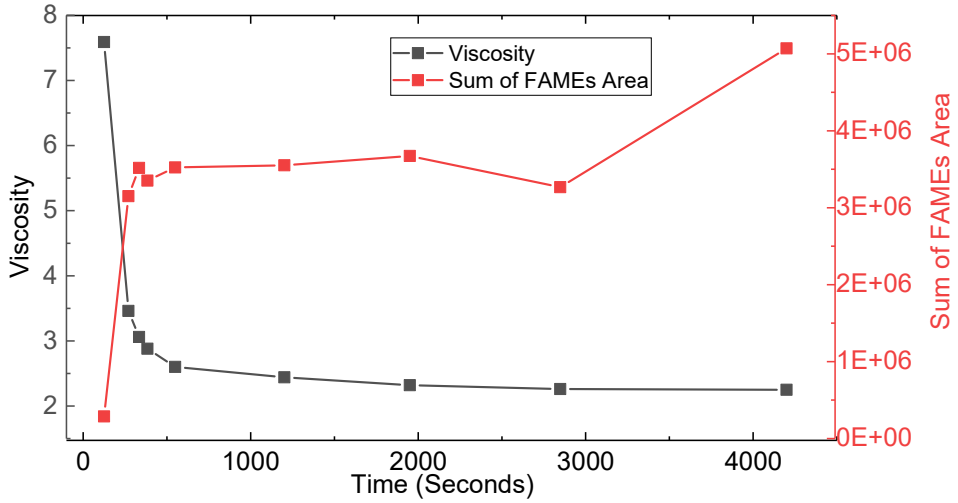


Figure 3.22. *Viscosity and FAMEs area change through time.*

Also in the case of the FAMEs productions the general trend is similar. However, in this case the methanol inside the reactor is less and the temperature is lower. This bring to a slower reaction velocity where the product of the TGs needs more time to become FAMEs.

It is possible to notice that when the reaction proceeds, the FAMEs are still produced. The values displayed in these figures are reported in Table 3.14. Also in this case it was calculated the real viscosity using the calibration curve (at 40°C) described in section 3.1.

Table 3.14. *Viscosity, Areas of FAMEs, Areas of TGs and conversion changes with time.*

Time (seconds)	Viscosity (mPas)	Real viscosity (mPas)	FAMEs Areas	TGs Areas	Conv. TGs
125	7.590	24.337	287175	3803314	44.6 %
271	3.460	9.056	3149776	1041174	84.8 %
334	3.060	7.576	3517468	227948	96.6 %
384	2.880	6.910	3353220	0	100 %
549	2.600	5.874	3523280	0	100 %
1201	2.440	5.282	3551584	0	100 %
1950	2.320	4.838	3672127	0	100 %
2848	2.260	4.616	3267382	0	100 %
4200	2.250	4.579	5071113	0	100 %

The results of this experiment are useful to understand that the behaviour of the viscosity during the transesterification is similar also if the reagent ratio or the temperature changes. However in this experiment the best conditions were not used, so the resulting FAMEs keeps increasing with the sampling time. This behaviour does not change the reliability of the monitoring process used, but suggest that, in this particular situation, the reaction should have been left going on for a longer time. Furthermore, the results are not completely trustfully because of the evaporation that occurs inside the samples while they were stocked inside the freezer. Really low temperatures help in fact the preservation of the solvents and the right concentration of the compounds, but in some cases the precautions used to seal the vials were not enough. This is the case of the sample taken at 2848 seconds in Table 3.14 for which it seems that the areas of the FAMEs decrease in relation to the sample taken before it. This sample should probably be considered as an outlayer because it is impossible that FAMEs decreases. Without that point the trend of FAMEs production could have been more reliable, explaining also the last point of the figure.

Despite the results of this part are not so good compared to the ones previously obtained at 50°C and r=6, the behaviour of the viscosity and its relation within the reagents and products of the reaction confirm almost the same behaviour. The general observed trend of curves is similar, even with drastic changes in operating conditions for the reaction.

This concludes the first part of the study on the monitoring method and the results allow to conclude that it can be extended to other conditions where not only the parameters are varied but different feedstocks are to be used.

3.5 Viscosity / FAMEs' area relation and fitting

So far, the viscosity was always analysed while changing with time. Taking into account the results and the chromatograms, it is also possible to find a relation between the viscosity and the inherent areas of the FAME's peaks. This particular relation suitably describes that the change of viscosity brings to a production of FAMEs and that a link between the two exists. Figure 3.23 describe the relation between the two variables in the first experiment (r=6 and 50°C) and in Figure 3.24 the relation in the case of the second experiment (r=4 and 40°C) is displayed. Since the viscosity in the first part of the reaction changes faster than in the second part, the points in the graphics are not regularly distributed. Nevertheless, it is possible to define an inverse relation between the two variables: the concentration of FAME increases approximately linearly as viscosity decreases.

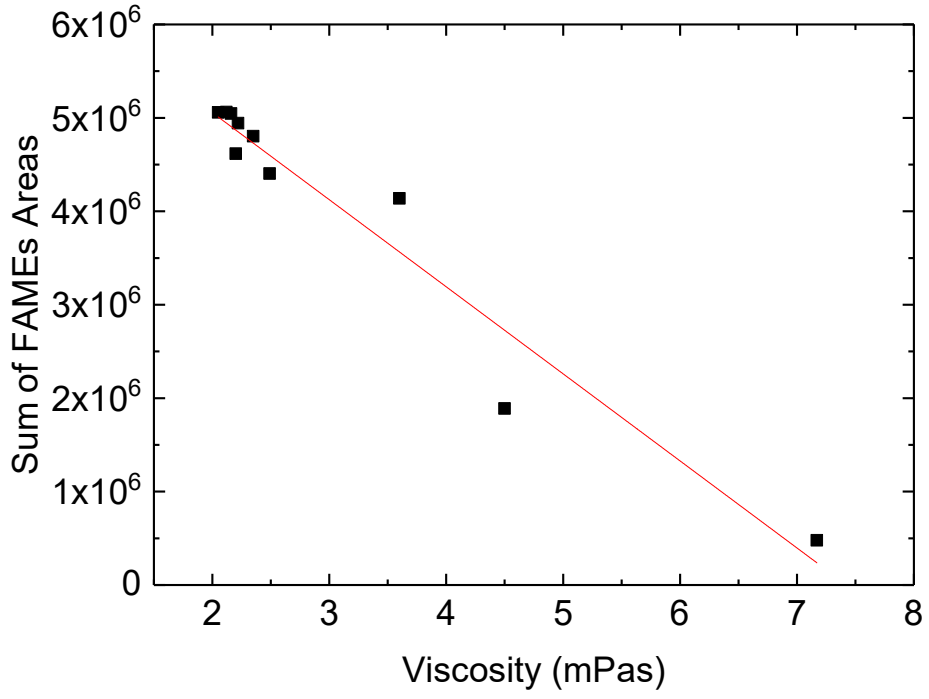


Figure 3.23. *Linear fitting viscosity FAMES' areas, reaction r=6 T=50°C.*

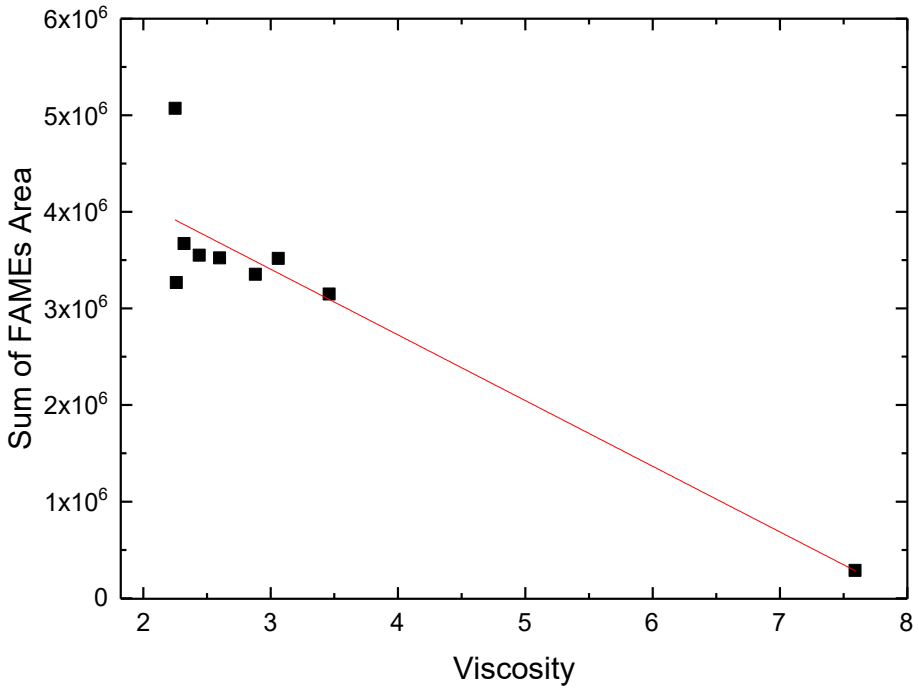


Figure 3.24. *Linear fitting viscosity FAMES' areas, reaction r=4 T=40°C.*

The fitting parameters of the linear relations displayed in Figure 3.23 and 3.24 are given in Table 3.15.

Table 3.15. *Fitting parameters of the linear relations displayed in Figure 3.23 and 3.24.*

	Intercept	**Slope**	**Adj. R^2**
Figure 3.23	6919960	-931912.15	0.93747
Figure 3.24	5446850	-680127.97	0.82592

The adjusted R^2 of the first experiment is better than the second one but the values shows that linear relations are reliable to derive the describe the reaction process. This is an ulterior confirmation of the assumption made in the previous paragraph: the first experiment's results are more reliable that the second one. Still, in the two experiments a relation between the FAMEs production and viscosity exist and can be found with this monitoring method.

Conclusions

In this thesis the on-line variation of the viscosity during biodiesel production from rapeseed oil in a small, laboratory, reactor have been analysed. It was then related the viscosity with the FAMEs production and TGs consumption with an HPLC analysis through time.
It was noticed that the forwarding of the reaction leads to a decrease of the viscosity. The graph showing the behaviour of the viscosity in function of consumption of TGs and FAMEs production show that former decreased while FAMEs were increasing, and TGs were decreasing. The conversion followed the same trend as the one of the FAMEs increasing fast at the beginning of the reaction till reaching a stable trend at the maximum reachable value.
The univocity of the viscosity and its trend in time has already been theorised and proved in laboratory, but always sampling and analysing the mixture outside the reactors. In this work, for the first time, it was proved that the trends found, following the FAMEs and TGs during the experiments, are analysable also on-line. The products and reagents can be determined without sampling and directly inside the reactor using a well calibrated viscometer.
All the products of the experiments were compared with an already commercialized biodiesel coming from PRIO.
Summarizing:

- An online, versatile method for the indirect determination of the products is proposed;
- This method can detect the reaction's end point in a simpler indirect way then the methods already used;
- The method proposed is based on the measuring of a physical property, more reliable and faster than the already used chemical analysis by chromatograph;
- The on-line method proposed is cheaper than the chemical one and can improve the safety of the plant, avoiding sampling of hot and dangerous compound from the reactors by the plant operators.

At this stage of the work, further investigations in a real plant should be conducted to assess the scalability and the practical feasibility of the monitoring method.

This thesis was performed during an ERASMUS+ period at the University of Coimbra.
The Portuguese company PRIO provided all the feedstock for the analysis and the reference biodiesel.

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Nomenclature

[n_D] = Refracting index

[u_s]= Speed of sound

[k]= kinetic constant

[Z]= Impedance

[ρ] = Density

[μ] = Dynamic viscosity

[υ] = Kinematic viscosity

[τ] = Shear stress

Units of measure

[Btu] =British thermal units

[Mtoe] =Million tons of Oil equivalents

[cP]= Centipoise

[RPM]= Ratio per minute

[Pa·s] = Pascal seconds

[N·s /m^2] = Newton seconds / square meters

Acronyms

DG= Diglyceride

EU= European Union

FAEE= Fatty acid ethyl esters

FAME= Fatty acids methyl esters

FFA= free fatty acids

G= Glycerol

GC= Gas Chromatography

HPLC= High performance liquid chromatography

HVO= Hydrogenated Vegetable Oils

IEO= International energy outlook

LCA= Life cycle analysis

LV= Uncertainty factor

M= Methanol

MG= Mono-glyceride

MIR= Mid-Infrared spectroscopy

NMR= Nuclear Magnetic Resonance

OECD = Organization for economic co-operation and development

PLS= Partial Less Square model

SCE= Size exclusion chromatography

TG=Triglyceride

US= United states of America

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Appendix A

While working in laboratory environment there are several attitudes that should be taken to ensure personal and collective safety. Safety standards where design to mitigate risks inherent in the use of dangerous reagents and potentially dangerous procedures. Any laboratory could represent a risky environment and the attitudes of those who work there can affect all the people that share the same space. There are several basic rules that should be always respect as the obligatory use of individual protection equipment like gloves and lab coat. Different procedures can dictate the need of other individual equipment like eye protection to ensure that no liquid get in contact with the eyes, hearing protection that when noise levels reach an unsafe level. For this project there are not high risks associated but some preventive procedures should be taken in account specially handling some compounds. Operation conditions are also not critical as all the steps are done under atmospheric pressure and transesterification occur at 50ºC which does not represent any special risk. The highest concerns are chemicals manipulation.

During laboratory tests some different chemical types were used, that included rapeseed oil, methanol, sodium methoxide, hexane (HPLC grade), 2-propanol (HPLC grade), acetonitrile (HPLC grade).

Globally harmonized system (GHS) is responsible for chemicals classification and labelling. Safety pictograms were created for people to know the main cautions and problems regarding a certain chemical Figure A.1. sums up the most used pictograms. In case of any accident that are some procedures that must be kept in mind to minimise the damage caused.

"*Check the vital functions. Unconscious: maintain adequate airway and respiration. Respiratory arrest: artificial respiration or oxygen. Cardiac arrest: perform resuscitation. Victim conscious with laboured breathing: half-seated. Victim in shock: on his back with legs slightly raised. Vomiting: prevent asphyxia/aspiration pneumonia. Prevent cooling by covering the victim (no warming up). Keep watching the victim. Give psychological aid. Keep the victim calm, avoid physical strain. Depending on the victim's condition: doctor/hospital. Never give alcohol to drink.*"

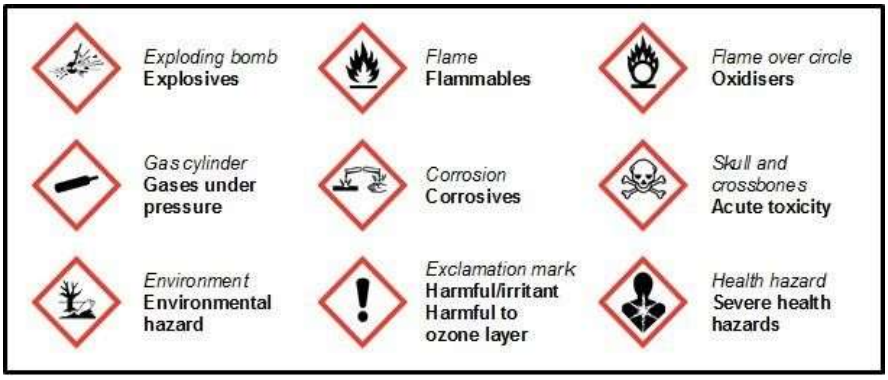

Figure A.1. *Illustration of the Pictograms used to represent chemical dangers*

Rapeseed oil

Rapeseed oil handling does not require any special attention since the only problem that can arise is flammability when used at really high temperatures, above 343ºC. Cotton seed oil should be storage in a ventilated place.

There are no effects known for eye contact but if it happens rinse with water for a few minutes should be enough.

When in contact with skin some irritation can happen. After contact with skin immediately wash with water is required and special attention to clean folds, crevices, creases and groin. A nonabrasive soap is the best choice to use during the washing. If there are any part of the skin exhibiting irritation an emollient shoe be used to cover the skin. If, the irritation persists, seek medical attention.

Methanol

Methanol manipulation requires special care since it is flammable and toxic and is it most always happen under a chemical fume hood. Hands should be washed after and before handling the product. Must be storage in a cool location provided with ventilation. Pictograms are indicated in Figure A.2.

Figure A.2. *Pictograms of the dangers regarding the handling of methanol*

In case of inhalation medical attention is required. Move the exposal individual to fresh air and loosen tight clothing such as a collar, tie, belt or waistband. Provide oxygen in case of any breath difficulty. After skin contact wash the affected area with water and soap for

approximately 15/20 minutes. Seek medical care in case of persistent irritation. A similar procedure is required in case of eye contact. Swallowing methanol also need medical assistance. After the episode rinse mouth thoroughly. The vomit should not be induced. Dilute mouth with water or milk after rising. In case of spilt use spark-proof tools to prevent explosion. Ventilation should be providing to the room and a non-combustible absorbent material should be used to contain the spilt.

Sodium methoxide

Sodium methoxide can represent dangers specially because it is corrosive. When working with sodium methoxide protecting clothes, gloves and glasses must be used. This reagent is extremely destructive to the tissue of the mucous membrane and upper respiratory tract. Sodium methoxide is not flammable or combustible. Pictograms are indicated in Figure A.3.

Figure A.3. *Pictograms of the dangers regarding the handling of Sodium Methoxide*

In case of inhalation and if the person is breathing just move them to into fresh air, if the person is not breathing perform artificial respiration and medical care is required. In case of skin contact, all the clothes must be removed. Wash off with soap and a lot of water, if needed, contact a doctor. In case of eye contact rinse thoroughly with plenty of water for at least 15 minutes and go to the hospital. If swallowed do not induce the vomit, rinse mouth with water and reach medical help.

Hexane

Hexane is a very dangerous chemical that can cause damages to organs due to prolonged and repeated exposure. Hexane target organs are liver, hearth and blood. Although there is no substantial proof it is suspected that it can affect fertility. Pictograms are indicated in Figure A.5.

Figure A.5. *Pictograms of the dangers regarding the handling of Hexane*

In case of inhalation the person must receive fresh air in a comfortable position for breathing. In case of contact with skin or hair, take all the clothes off and rinse the skin with water. Wash contained clothes before use again. In case of eye contact rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. If the irritation persists, contact medical support. In case of fire: Use CO2, dry chemical, or foam for extinction. If swallowed rinse mouth and do not induce vomit, medical care is needed. The place where hexane is storage must be locked up and ventilated. Any residues resulting from hexane handling must be disposed to an approved waste disposal plant. Hexane is very toxic, and it can have long term effects in aquatic life.

Acetonitrile and 2-propanol HPLC grade

Acetonitrile and 2 propanol are used during HPLC analysis and it is not directly handling during any procedure since it is in the container that is connect to the HPLC column. The first one is a solvent and the second an alcohol. The most concerns with this chemical are its flammability in liquid and vapour state and irritation in the eyes. For this reason, acetonitrile must be kept away from heat, sparks, open flames and hot places. In case of inhalation, skin or eye contact or swallowed take the measured referred above for hexane. Pictograms are indicated in FigureA.6.

Figure A.6. *Pictograms of the dangers regarding the handling of Acetonitrile and 2-propanol*

Appendix B

In this appendix are reported the chromatograms of all the sample analysed from which the value in Table 3.11 and 3.14 comes from.
All this samples were analysed using a HPLC C18 reverse column, and in the same way described in the second chapter.

First reaction r=6 T=50°C

Sample 2

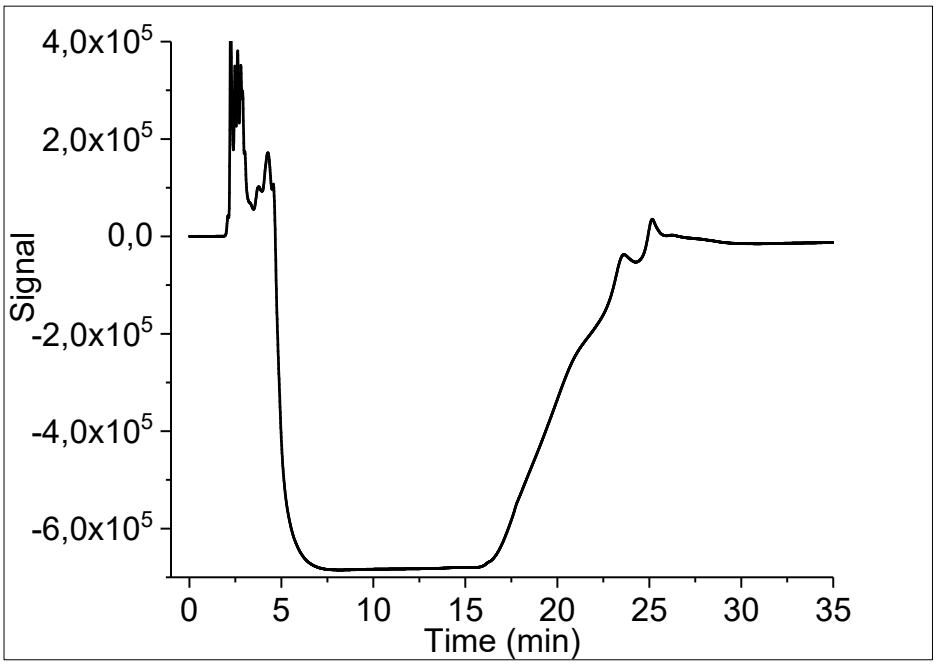

Figure B.1. *Second sample t=129 sec*

Sample 3

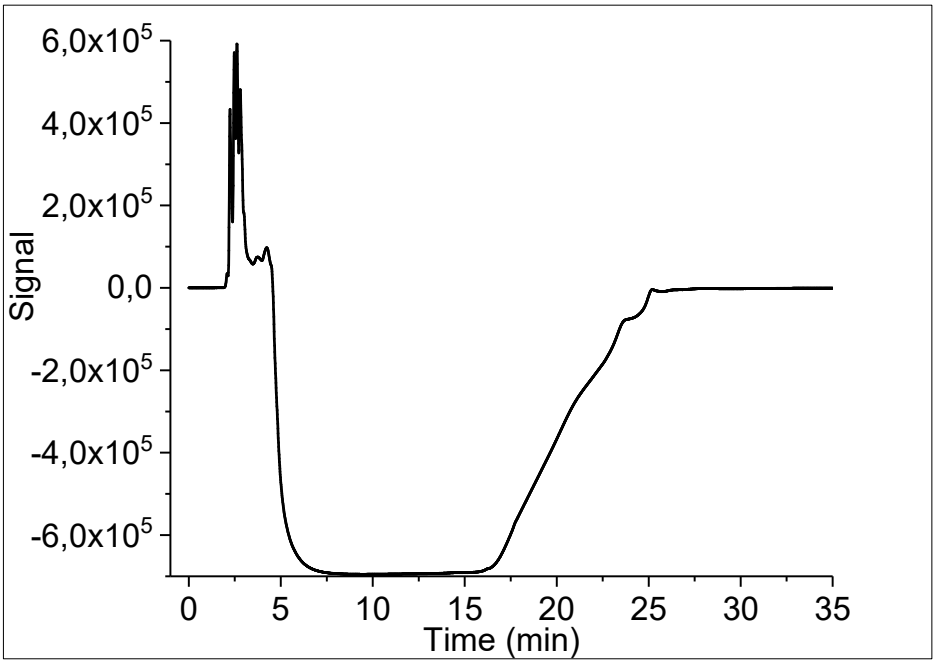

Figure B.2. *Third sample t=194 sec*

Sample 4

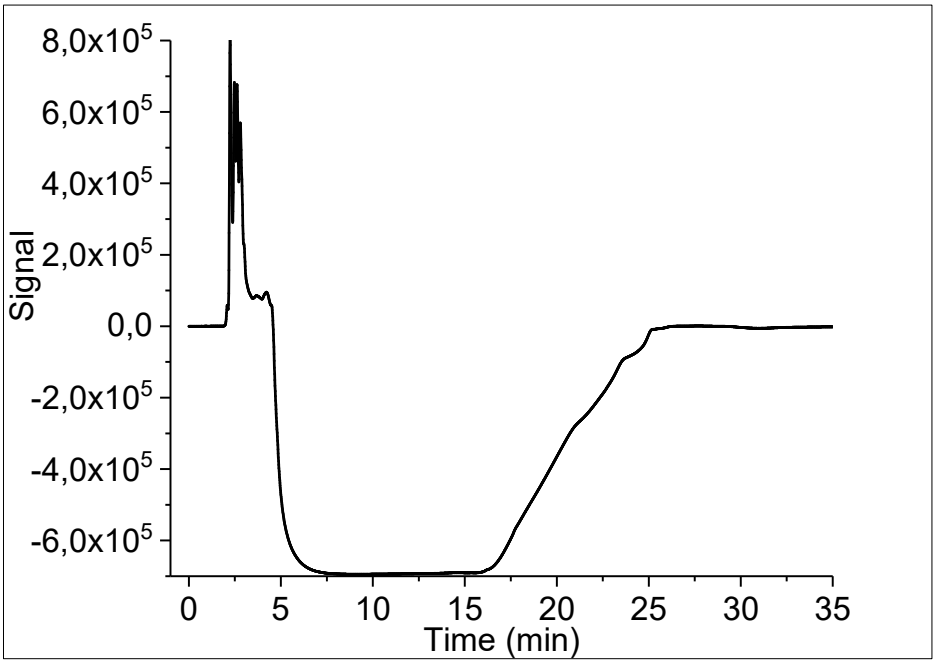

Figure B.3. *Fourth sample t=273 sec*

Sample 5

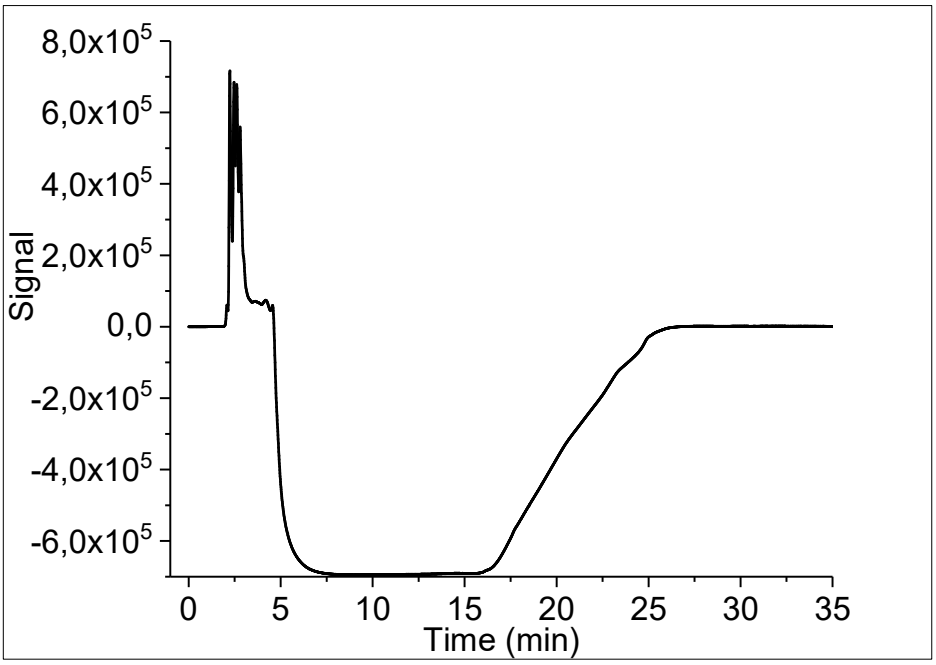

Figure B.4. *Fifth sample t=501sec*

Sample 6

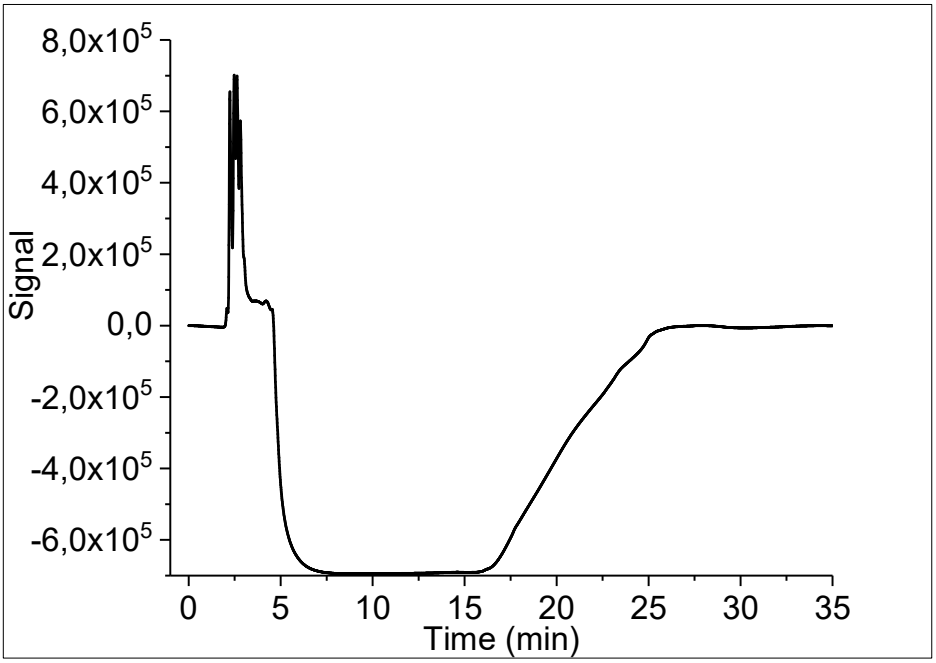

Figure B.5. *Sixth sample t=684 sec*

Sample 7

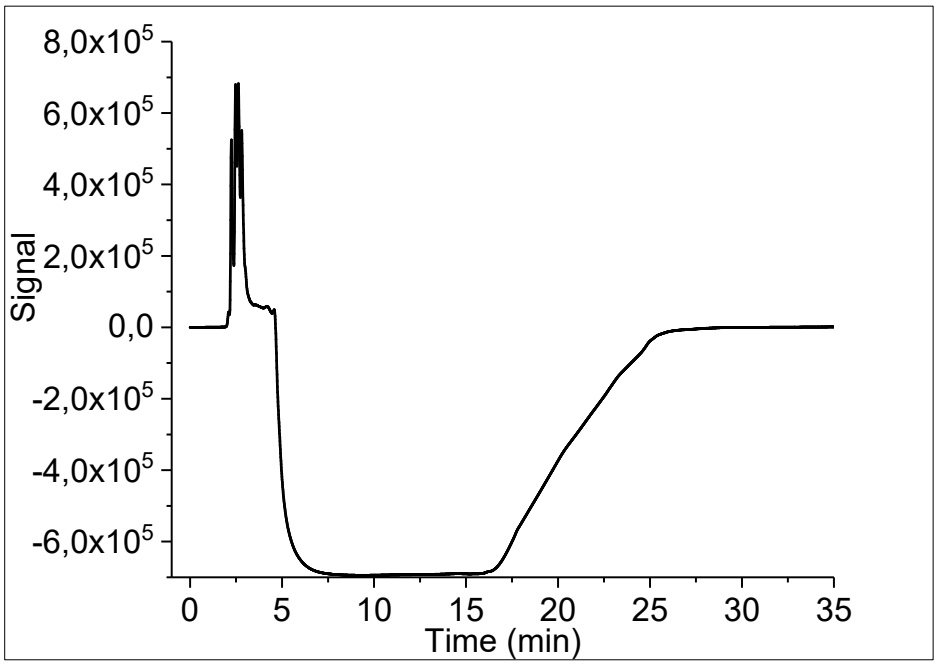

Figure B.6. *Seventh sample t=1320 sec*

Sample 8

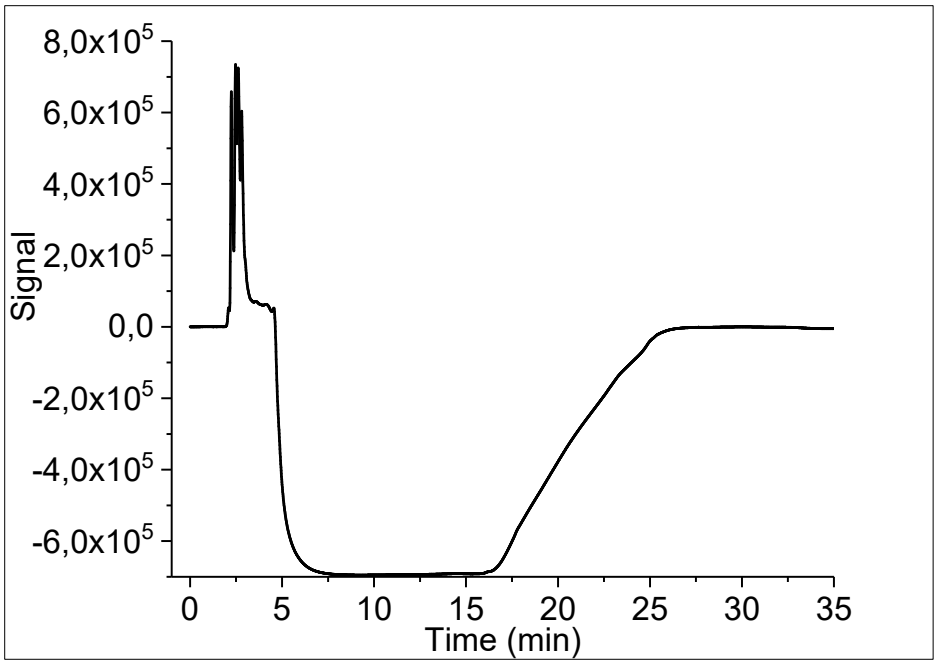

Figure B.7. *Sixth sample t=1986 sec*

Sample 9

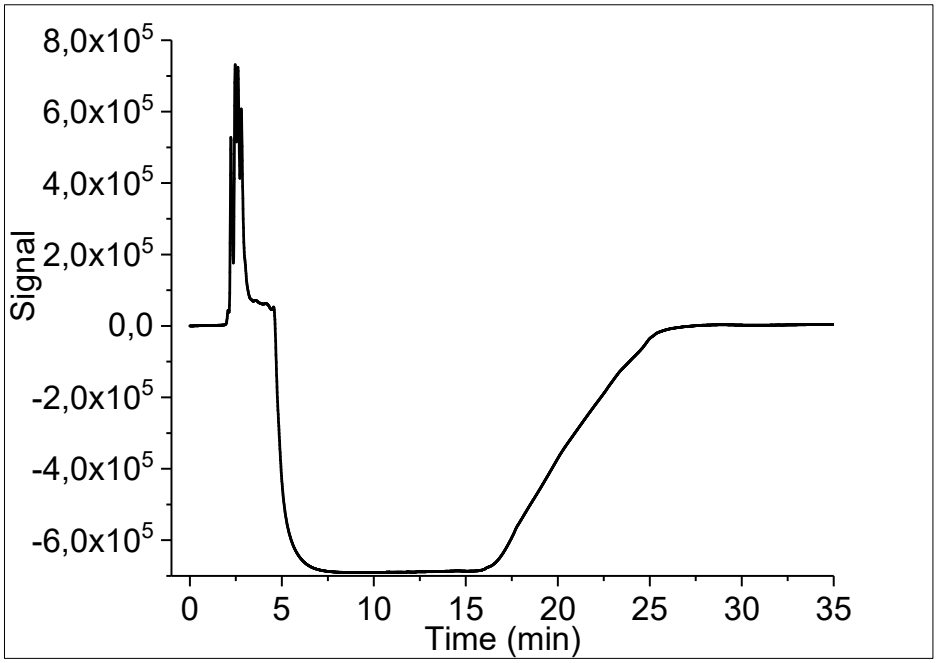

Figure B.8. *Ninth sample t=2657 sec*

This are all the sample of the first reaction performed at the best condition. It is possible to confirm all the discussion done since now. The decreasing of the TGs peaks and the increasing of FAMEs are detectable through time.

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