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Fish oil chemical composition for biodiesel production

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Abstract

Here we report a study of oil fish properties that can be used for biodiesel production. The objective is to choose the suitable oil that allows the production of biodiesel with improved rheological properties and a good oxidation stability. These properties are influenced by the unsaturation degree, the chain length of fatty acids and the percentage of oleic, erucic and palmitic acid. Fish oil is assimilated as an alternative to fossil fuels. The oils used are extracted from three different by-products (salmon, trout and sardine by-products). The results of this study show that among the studied oils, salmon oil is more stable thanks to its abundance of short-chain fatty acids, its oleic acid richness and erucic acid poverty. These properties allow to salmon oil to be the most suitable oil for biodiesel production.

1. Introduction

Energy demand will continue to increase significantly in the coming years due to population growth and the gradual rise in living standards, particularly in developing countries. Thus, needs are expected to double by 2050. To meet this demand, energy sources will become more complementary than competitive. All energy options must be kept open in order to provide the most appropriate responses, both environmentally and economically.

The depletion of the world's oil resources and the intensification of laws and regulations related to their exploitation are directing scientific research towards new sources of energy. Renewable energies have attracted a lot of interest as an alternative energy source. The use of nuclear energy as well as other renewable energy sources such as photovoltaic and thermal solar panels and wind turbines is limited to the production of electricity and heat. These sources don't have enough capability to replace the petrol. Therefore, it is recommended to find an alternative fuel to fossil diesel. Biofuels are renewable, non-toxic and can be produced locally from agricultural resources and slaughterhouses. In addition, they emit fewer harmful emissions into the environment. It is therefore interesting to use an energy source while preserving the environment.

The increase in fish processing waste and the expansion of the renewable energy market imply that fish processing waste could be important in the biofuel future[1]. The valorization of this biomass is an important issue to allow a more profitable and sustainable exploitation of the marine resource. For this valorization such as the oil extraction from fish by-products, environmentally friendly soft technologies like reactive extrusion have been used. Significant results have been obtained compared to other technologies like batch process [2]. Fish processing waste rich in fat by extracting fish oil can be used for biodiesel, although it can be mixed with diesel while complying with biodiesel standards[3,4].

The biodiesel fatty acid profile is the same as that of the parent oil and it's a major factor influencing the fuel properties [5–8]. The properties influencing the physical characteristics of the biodiesel produced are the unsaturation degree, the chain length and the percentage of oleic, erucic and palmitic acid [8–11]. The main properties of biodiesel as a fuel that are influenced by the fatty acid profile are exhaust gas emissions, combustion heat, cold flow, melting temperature, oxidative stability, viscosity and lubricity. Thus, for the use of oils in biodiesel production, it's essential to identify their different characteristics and compare them in order to choose the most appropriate oil for such use.

2. Material and Methods

2.1. Raw material

The oils used are salmon, trout and sardine oils that are extracted from fish by-products (head, skin and bone of fish). The extraction process is reactive extrusion process. It is a complex process covering two distinct fields, chemistry and conventional extrusion, in a single operation, where the extruder is assimilated to a continuous type chemical reactor. The extruder is used in different fields in particular the chemistry field. The researches have led to the development of new materials such as flame retardant materials [12] and bio-sourced materials [13]. The process has used also for the bio-substances extraction (chitin, alginate, fish oil) from seafood products and it has shown positive results compared to other traditional extraction processes [2, 14–16]. The oils are obtained by enzymatic reaction of fish by-products by using the extruder. The fish by-products are introduced in the extruder where they undergo an enzymatic reaction by adding a protease-type enzyme. Due to the strength of the extruder and the enzyme effect, the process allows to extract lipids from fish by-product matrices.

2.2. Profiling of total fatty acids by gas chromatography-mass spectrometry (GC-MS)

The profiling of different fatty acids contained in fish oils is identified by Gas Chromatography (GC). It is an analytical method used to determine the fatty acid composition of oils.

The analysis of fatty acids is done in 3 steps: extraction of lipids, transmethylation of total lipids to make them volatile for GC profiling. Weigh a mass of approximately 10 mg of sample into an 8 ml glass vial, previously burned at 450°C for 6 hours, then add 8 ml of a mixture of CHCl3/MeOH (2/1-v/v) to extract the lipids. Then, 600 µL of this mixture is sampled and diluted in 6mL of CHCl3/MeOH (2/1-v/v). The tube is left on a pendulum for 6 hours to fully extract the total lipids. The extract is then evaporated dry and added in order: 40µL of TG17:0 (internal standard, 35.58µg) and 1.2 mL of boron trifluoride (BF3, 14% by weight methanol). Transmethylation is then performed for 10 minutes at 90°C. After cooling, a volume of 1.5 mL of hexane is added to recover total fatty acids (TFA). The organic phase containing fatty acid methyl esters (FAME) is washed 3 times with 2 mL of water saturated with hexane. After washing, the solution containing the FAME, hexanic phase, is analyzed by gas chromatography with a 7820A chromatograph (Agilent Technologies, Santa-Clara, CA, USA) composed of a split/splitless injector (250°C, injected quantity: 2µL, split set to 1/10 -10 mL/min purge), with a constant flow (hydrogen) at 1mL/min. The carrier gas is produced by a hydrogen generator (WM-H2, F-DGSi, Evry, France). The column used for separation is a TR-FAME capillary column (very polar phase 70% Cyanopropyl Polysilphenylene-siloxane, inner diameter 0.25 mm, thickness 0.25 µm, length 30 m, Thermo Fisher Scientific, Whaltham, MA, USA). The temperature of the chromatography furnace is set at 80°C for 1 minute, then increases at a rate of 5°C/min to 145°C for 12 minutes, then increases further at a rate of 1°C/min to 155°C for 5 minutes, then increases at a rate of 5°C/min to 200°C for 5 minutes. The detector used is a flame ionization detector maintained at 280°C, with hydrogen and air flows maintained at 30mL/min and 300 mL/min respectively. The identification of FAMEs is done by comparing their retention time with that of the standards used for calibration. The calculation of the concentrations of each FAME is performed using Chemstation v 0.1.0.4 with TG17:0 as the internal standard.

2.3. Identification of Triglyceride rate by high performance thin layer chromatography (HPTLC)

The different lipid classes can be determined by high performance thin layer chromatography (HPTLC). We are interested in neutral lipid classes: to know the amount of free fatty acids (FFA), triglycerides (TG), glyceride ethers. The sample was extracted in the same way as for the above-mentioned KM protocol. The extracts are analysed using the CAMAG system (GAMAG, Muttenz, Switzerland) equipped with modules for activating chromatography plates (20*10cm silica gel plate 60 (Merck 1.05642.0001)), plate migration (20*20cm tanks), automatic sample deposition (Automatic TLC Sampler ATS4), revelation (Chromatogram Immersion Device) and densitometry quantification (TLC Scanner 3). The software that drives the different modules is WinCats software. The deposits are made on silica plates which are previously thermally activated (30 min at 120°C) after a first blank elution (hexane/diethyl ether/acetic acid 20/5/0.5-v/v/v/v). The compounds separation is carried out with a mixture of hexane/diethyl ether/acetic acid eluent 20/5/0.5-v/v/v/v over a total migration length of 6 cm. Then a second elution with hexane/diethyl ether (97/3-v/v) over a total migration length of 7 cm separates the neutral lipid classes of interest. The compounds are detected by densitometry (371 nm) after detection with a solution of phosphoric acid and copper sulphate (16g H3PO4+ 6g CuSO4+ 200mL H2O distilled) (30 min at 180°C). Each component has a standard molecule that optimizes separation and determines its concentration.

3. Results and discussion

3.1. Unsaturation degree Comparison of the three types of oils

3.1.1. Salmon oil

Salmon oil is an oil containing 92% unsaturated fatty acid and 8% saturated fatty acid. The unsaturated fatty acids contained are divided into 57% monounsaturated and 35% polyunsaturated. The main monounsaturated fatty acids identified are oleic acid (C18:1n-9), 11-eicosenoic acid (C20:1n-9) which are classified among the fatty acids of the omega-9 series and palmitoleic acid (C16:1n-7) which is the most common omega-7 fatty acid (Figure 1).

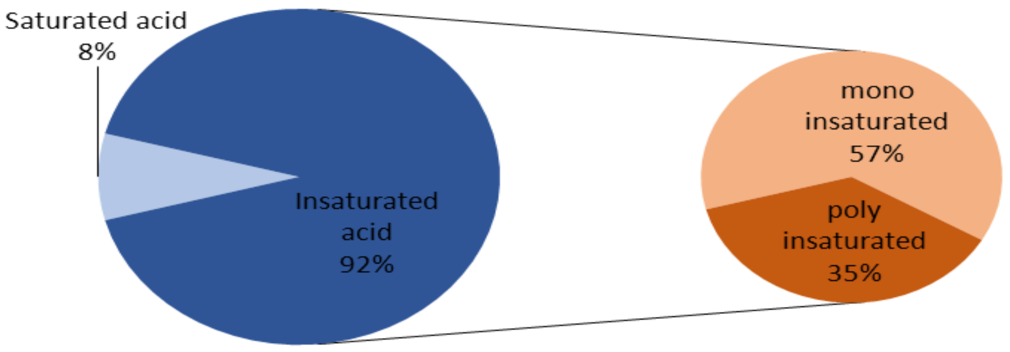

Figure 1: Saturated and unsaturated fatty acids in salmon oil

3.1.2. Trout oil

Unsaturated fatty acids are the major acids in trout oil composition with a percentage of 90%, while saturated fatty acids represent only 10%. Monounsaturated fatty acids constitute 58% of total unsaturated fatty acids, of which oleic acid, which is an acid of the omega-9 series, represents a large percentage of 49%. Gadoleic acid and erucic acid classified as omega-9, represent percentages close to 2.60% and 2.92%, respectively (Figure 2).

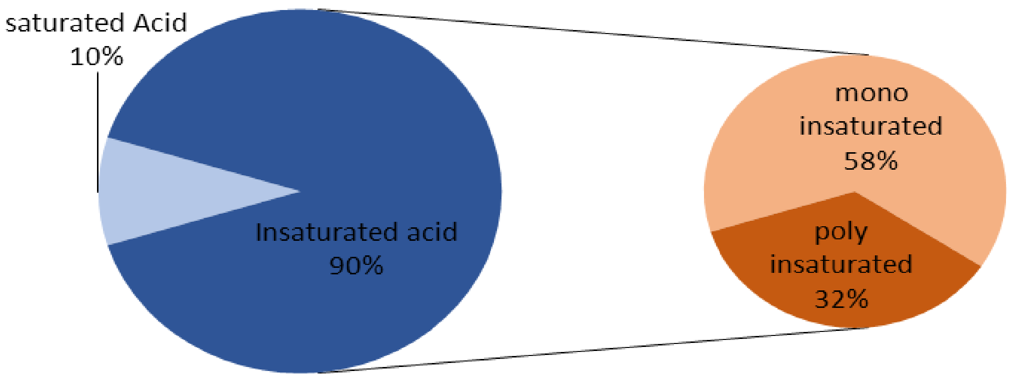

Figure 2: Saturated and unsaturated fatty acids in trout oil

3.1.3. Sardine oil

Of the 69% of unsaturated fatty acids in sardine oil, 51% are monounsaturated and the remaining 18% are polyunsaturated (Figure 3). The major mono-unsaturated fatty acids distinguished are acids from the omega-9 series such as erucic acid (C22:1n-9), oleic acid (C18:1n-9), nervonic acid (C24:1n-9) and 11-eicosenoic acid (C20:1n-9). The other fatty acids identified are palmitoleic acid (C16:1n-7) and myristoleic acid (C14:1n-5) which are acids belonging to the omega-7 and omega-5 series, respectively.

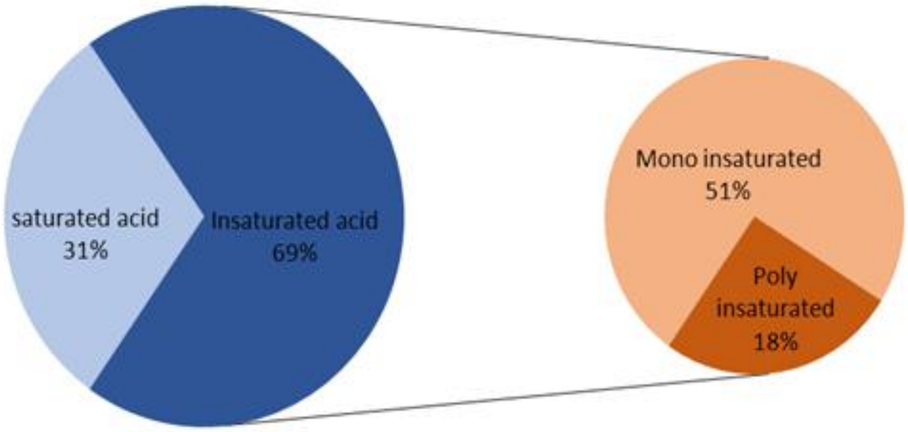

Figure 3: Saturated and unsaturated fatty acids in sardine oil

By comparing the three types of oils, we can identify the abundance of unsaturated fatty acids in salmon oil and trout oil. Unsaturated acids also have a better lubricating power than saturated acids. Therefore, the use of salmon and trout oils is recommended for the biodiesel production.

The monounsaturated acids rates in the three oil types are almost similar. The degradation metabolic pathway of these fatty acids, which depends on the Omega series to which they belong, is different for each type of oil. Most of the monounsaturated acids found in salmon oil and trout oil belong to the omega-9 series. On the other hand, the monounsaturated acids contained in sardine oil are divided into omega-9 and omega-7. Thus, based on the above results, it is concluded that salmon and trout oil are the most appropriate for biodiesel production with improved properties.

The oil unsaturation degree is an essential parameter for its use in biodiesel production. The content of unsaturated and saturated fatty acids has an effect on the lubricity and also on the oxidation stability of the biodiesel produced. Unsaturated acids have a better lubricating power than saturated acids [17]. Saturated fat compounds have higher melting points than unsaturated fat compounds. Biodiesel derived from oils containing saturated fatty compounds, which are in the majority, has a low fluidity at low temperature indicated by their relatively high cloud point and pour point [17]. The pour point, which usually occurs at a temperature lower than the cloud point, is the minimum temperature at which the product still flows. The cloud point is the temperature at which a liquid fat becomes cloudy due to the crystals formation and saturated fat solidification. Solids and crystals form quickly and clog fuel lines and filters, causing significant operational problems [18]. Therefore, the biodiesel cold flow parameters are determined by the amount of saturated fatty acids with a high melting point, regardless of the unsaturated fatty acids nature [19].

The unsaturated fatty acids have lower melting points, which is recommended for improved biodiesel properties at low temperatures, but also have reduced oxidation stability, which is not desirable for diesel fuel. The reason for auto-oxidation is the presence of double bonds in the chains of many fatty compounds. The unsaturated fatty compounds auto-oxidation continues with different rates depending on the number and position of the double bonds. Thus, among the unsaturated acids, it is recommended to have a high content of monounsaturated acids, although they provide the biodiesel cold flow conditions and at the same time a good oxidation stability compared to other unsaturated fatty acids [20]. The double bonds position has a major effect on the fatty acids degradation and, consequently, on the oil stability. Unsaturated fatty acids can be classified, depending on the unsaturation position in the chain, into different series. These series are called "Omega-n", n represents the position of the first unsaturation from the side opposite the acid group, the higher n is, the better the unsaturated fatty acid is less susceptible to degradation.

The suitable oil choice for biodiesel production is therefore based on its content of saturated and unsaturated fatty acids, particularly mono unsaturated acids.

3.2. Chain lengths Comparison

The length of the fatty acid chains influences the biodiesel produced properties. Melting temperature and viscosity are fuel properties that indicate that fatty compounds are suitable as diesel fuel [21]. These factors increase with the fatty acid chain length [17]. The viscosity factor makes biodiesel sensitive to temperature and pressure variations. As well as, the fatty acid melting temperature influences the biodiesel cold flow [8].

Salmon oil contains mainly long chain fatty acids of a percentage of 91.95% (Figure 4-a). The short chain fatty acids represent a small percentage of 8.05%. Figure 4-b shows the percentages of long-chain and short chain fatty acids in trout oil. The short chain fatty acids represent a percentage of 83.51%, which is more interesting than long chain fatty acids which represent only 16.49%.

As shown in Figure 4-c, the proportions of fatty acids contained in sardine oil have a relatively similar chain length, which represents approximately 50%. Hence the percentages of short-chain and long-chain fatty acids are close.

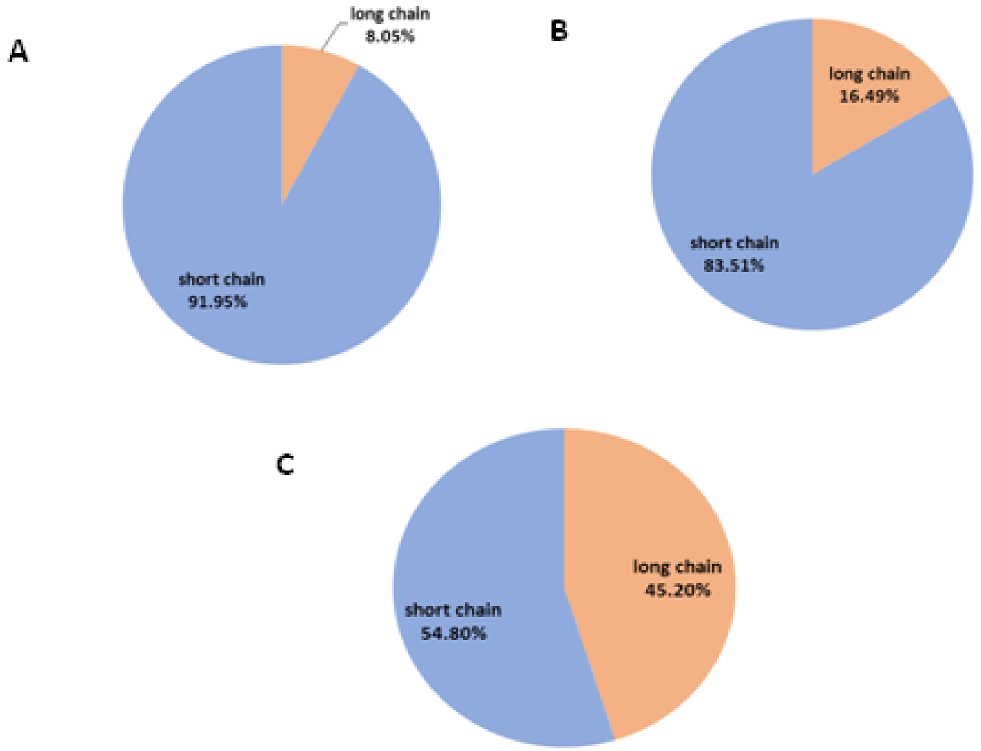

Figure 4: Short and long chain acids percentages in: (a) salmon, (b) trout and (c) sardine oils

Comparison of fatty acid profiles in the three types of oils shows large short-chain fatty acids predominance in salmon and trout oils. Thus, for biodiesel production at a low melting temperature while reducing its viscosity, salmon and trout oils are the most appropriate for this purpose.

3.3. Oleic and erucic acid rates

Most biodiesel fuels contain significant amounts of esters of oleic, linoleic or linolenic acids, which influence the oxidation stability of the fuels. Knothe [7] conducted a study to improve the biodiesel physico-chemical properties such as oxidation stability, cold flow, viscosity and cetane number. Shorter-chain fatty acids than oleic acid have a low cetane number problem. On the other hand, fatty acids with a longer chain than oleic acid are problematic because of their high melting temperature, which represents an obstacle during cold flow [18]. Increasing oleic acid in the fatty acid profile has been the most common approach to balance oxidation stability and cold flow while keeping the cetane number at an acceptable value [11]. The melting point of the oleic acid methyl ester at -20°C would be sufficient for most cold climate conditions.

Standard oxidation measurements show that products with a high oleic acid content are much more stable to oxidation than products with a low oleic acid content. As well as, during engine emission tests, this high stability of oils with a high oleic acid content makes it possible to obtain a significant reduction in NOx emissions during combustion [22].

In order to obtain an even better lubricating power than that usually observed with soybean oil-based biodiesel while preserving oxidative stability, Kinney and Clemente [6] suggested enriching ricinoleic acid with oleic acid. Ryan et al. [23] studied the consumption rates of biodiesel extracted from different types of oils. Indeed, they showed that the mixture of diesel and groundnut oil is low in consumption compared to cottonseed oil. This is probably due to the richness of peanut oil in oleic acid compared to cottonseed oil. It is interesting to note that the use of an oil rich in oleic acid has an effect on the consumption rate of the biodiesel produced.

The erucic acid melting temperature is higher than that of oleic acid. A high melting temperature influences the cloud point resulting in the solid particles formation, which clogs the pores of the fuel filters. Regarding viscosity, oleic acid esters are less viscous than erucic acid esters. As well, the boiling temperature of erucic acid esters is higher than that of oleic acid. A high boiling point means that biodiesel tends to partially vaporize, forming deposits on engine components.

In the literature, a comparative study was carried out between the transesterification of the same type of oil with a high erucic acid content and a low content for the biodiesel production. The comparison showed a difference between high and low erucic acid oil in terms of transesterification efficiency, viscosity and properties of the biodiesel produced. The transesterification efficiency of high erucic acid oil is low due to soap formation, which results in a minimum ester yield. In addition, the kinematic viscosity of high erucic acid oil is higher than that of the same type of oil but does not contain erucic acid. In addition, biodiesel from oil with a low erucic acid content has the same properties as diesel [24].

In order to select the appropriate oil for biodiesel production based on oleic and erucic acid levels, a comparison was made between the three types of oils. Figure 5 shows the different percentages of oleic and erucic acid in the three types of oils.

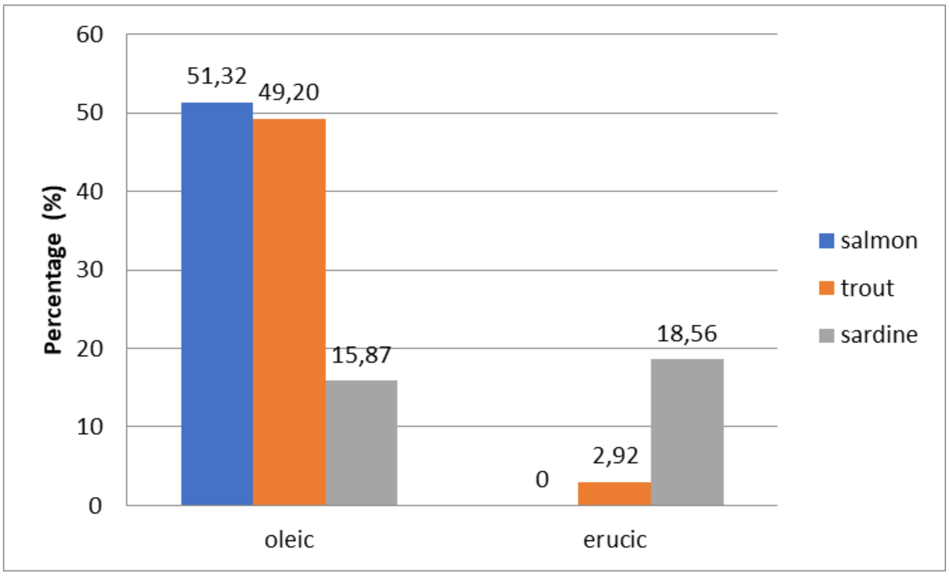

Figure 5 : Percentages of oleic and erucic acid in the three types of oils

Salmon oil is characterized by a high percentage of oleic acid of 51.32% followed by trout oil of 49.20%. Sardine oil represents the lowest percentage of 15.87% oleic acid. For the erucic acid content, sardine oil is the richest in erucic acid with a percentage of 18.56%. Trout oil has a low erucic acid content of 2.92%. However, salmon oil does not contain erucic acid.

3.4. Oleic and palmitic acid rates

The presence of oleic and palmitic acid in different proportions in the oil influences the biodiesel produced properties. Indeed, the high oleic acid content has a positive effect on the biodiesel properties. These properties

are more improved in the presence of a low palmitic acid content. Oil with a high oleic acid content and a low palmitic acid content provides high oxidation stability, which reduces NOx emissions during combustion [6].
The rates of these two acids have been determined in all three types of oils to designate the oil that produces a biodiesel with improved properties. As illustrated in Figure 6-a, oleic acid represents half of the total fatty acids in salmon oil. The second half is divided into a low percentage of palmitic acid of 3% and a rate of 46% including the other fatty acids contained in the oil.
In trout oil, oleic acid is abundant, representing 49% of total fatty acids. Then the other existing fatty acids represent a percentage of 51% of which a low rate of 4% is represented by palmitic acid (Figure 6-b). Figure 6-c shows that oleic and palmitic acids are present in sardine oil in low concentrations. They represent a percentage of 26% which is divided into 16% for oleic acid and 10% for palmitic acid.

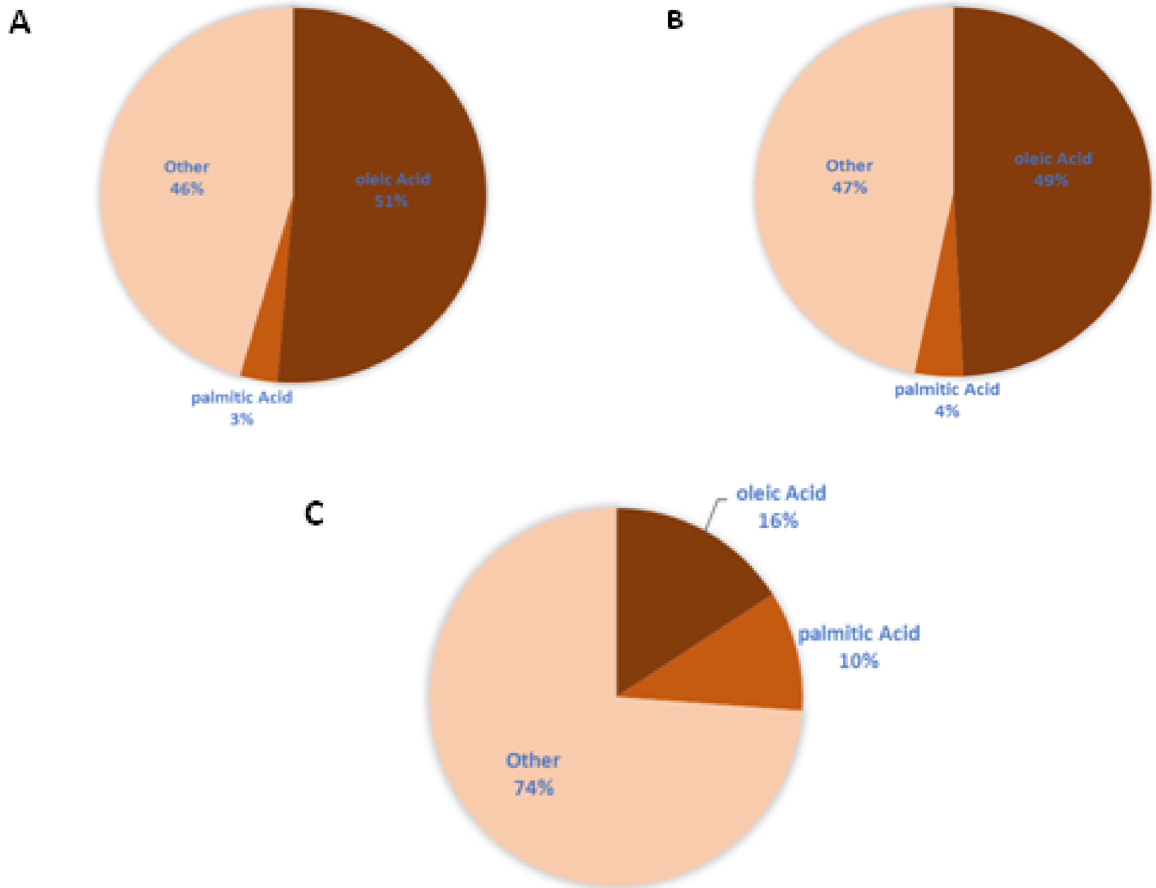

Figure 6: Oleic and palmitic acid distribution in (a) salmon, (b) trout and (c) sardine oils

According to the above statistics, salmon and trout oils are among the high oleic acid and low palmitic acid oils. Oleic acid accounts for almost half of the two types of oils mentioned above and palmitic acid for a small percentage. These fatty acid profiles allow the production of a biodiesel that is more stable to oxidation during storage. Biodiesel oxidation can lead to increased acidity index and viscosity, and the appearance of sediments and crystals that clog filters. When the physico-chemical properties of biodiesel, such as acid number, viscosity and sediment measured, exceed the limits of ASTM D6751, biodiesel becomes out of specification and cannot be used as a fuel.

3.5. Triglyceride rate

For the biodiesel production, it is necessary to transesterify the oil. A triglyceride content identification for each oil is recommended to have a good yield in biodiesel production. The HPTLC analysis results showed a difference in the lipid classes contents, in particular those of triglycerides, for each type of oil studied. In addition, salmon oil is determined to be the richest in triglycerides with a level of 1391.6 µg/ml, followed by sardine oil and trout oil with a level of 1046.5 µg/ml and 214.1 µg/ml, respectively (Figure 7).

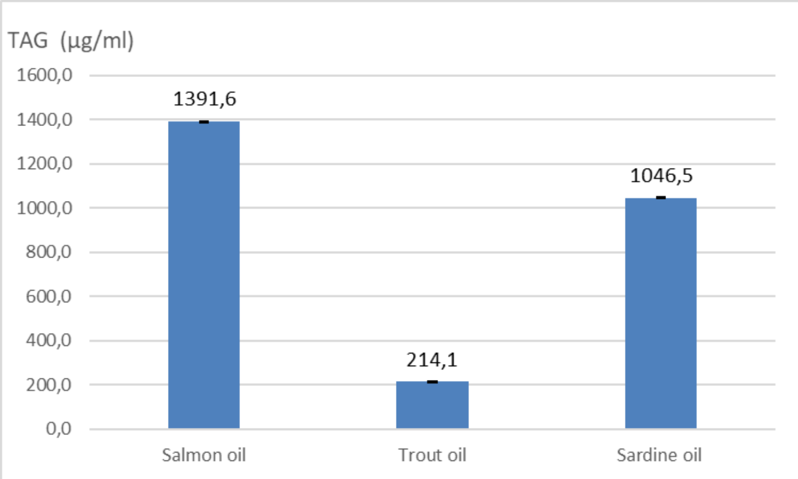

Figure 7: Triglyceride rates in the three types of oil

Conclusion

The biodiesel produced properties are influenced by the mother oil used. Each type of fish oil (salmon, trout and sardine oil) has its own chemical characteristics. These characteristics influence its cold flow, melting temperature, oxidative stability, viscosity and lubricity. Chromatographic analysis identified the richness of salmon oil in short chain and insaturated fatty acids, 91.95% and 92% of total fatty acids respectively. The monounsaturated fatty acids represent 57% of unsaturated acids found in this oil, and they belong to the omega-9 series. These proportions allow to salmon oil to have a better lubricity and a higher oil oxidation stability compared to other oils. The high short chain fatty acids rate allows the production of biodiesel at low melting temperature and viscosity. This oil has improved rheological properties in cold flow.

The oleic, palmitic and erucic fatty acid rates impact the oil characteristics. Unlike trout and sardine oil, salmon oil is rich in oleic acid (51% of total fatty acids), low in palmitic acid (3% of total fatty acids) and does not contain erucic acid. These characteristics make this oil more stable than the other oils studied. As a result, the efficiency of its transesterification is more profitable and the production of biodiesel is more favourable since its physico-chemical characteristics are similar to those of diesel.

The transesterification reaction is important to produce biodiesel from oil. Therefore, triglyceride rate influence on the reaction yield. Compared to other oils, salmon oil contained an important triglyceride rate which represents 1391.6 µg/ ml.

After this study, the optimization of the transesterification reaction will be imposed in order to produce a biodiesel with characteristics similar to those of diesel and complying with the standards.

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