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# Master thesis

Matter sciences
Chemistry
Pharmaceutical chemistry

Ref:

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The : July 8, 2021

# Characteristics of linseed oil

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Academic year: 2020/2021

لرُفاَتِكَ أبي و دَمعِكِ أمي

أهديكما سطوراً علمني الله إياها

مذكرتي


حَسَدوا الفَتى إِذ لَم يَنالوا سَعيهُ
فَالقَومُ أَعداءٌ لَهُ وَخُصومُ
كَضَرائِرِ الحَسناءِ قُلنَ لِوَجهِها
حَسداً وَبَغياً إِنَّهُ لَدَميمُ
وَالوَجهُ يُشرُقُ في الظَلامِ كَأَنَّهُ
بَدرٌ مُنيرٌ وَالنِساءُ نُجومُ
أبو الأسود الدؤلي

# Abbreviations

| | |
|---|---|
| **EV:** | Ester value |
| **SV:** | Saponification value |
| **AV:** | Acid value |
| **A%:** | Acidity |
| **POV:** | Peroxide value |
| **pH:** | Potential hydrogen |
| **$H^1$ NMR:** | Proton nuclear magnetic resonance spectroscopy |
| **FTIR:** | Fourier transformation infrared spectroscopy |
| **IR:** | Infrared spectroscopy |
| **DI:** | Degree of isomerization |
| **KOH:** | Potash |
| **ICl:** | Wijs reagent |
| **KI:** | Potassium iodide |
| **$Na_2SO_4$:** | Sodium thiosulfates |
| **L1:** | 100 ml of used linseed oil |
| **L2:** | 1g of Gallic acid /100 ml of used linseed oil |
| **G1:** | 0.1g of Gallic acid /100 ml of used linseed oil |
| **G2:** | 0.3g of Gallic acid /100 ml of used linseed oil |
| **G3:** | 0.7g of Gallic acid /100 ml of used linseed oil |

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# General introduction

# General introduction

Flax (Linum usitatissimum L.) is one of the most important oil plants in the world, especially in Canada and China. In China, flax can be mainly divided into two types, namely oil-flax and fibre-flax, according to their use. Oil-flax is planted for oil from the seeds and mainly distributed in northwest of China. Fiber-flax is grown for fiber from its stem and mainly distributed in northeast of China. The seeds of both the oil-flax and fiber-flax can be used to obtain oils. Flax seed, also known as flaxseed or linseed, contains 20–40% oil **[1–3].**

Flaxseed oil is a typical drying oil and mainly used for industrial purpose, such as the production of paints, linoleum, varnishes, inks, and cosmetics, in the past. However, flaxseed oil is becoming popular for its nutritional and pharmaceutical values.

Flaxseed oil is known as the richest source of the n-3 fatty acid, alpha linolenic acid (ALA), which is one of the essential fatty acids **[4].**

Studies have proven that flaxseed oil has positive effect on many diseases, such as hyperlipidemia, **[5]** colon tumor, **[6]** mammary cancer, **[7,8]** and atherosclerosis **[9,10]**.

Previous studies on flaxseed oil were mainly based on the industrial purpose, such as oxypolymerization **[11, 12]** and oxidative degradation **[13].**

Before flaxseed oil is introduced in the food field, its characteristics must be realized. Wiesenborn and al. **[14]** have studied the sensory and oxidative quality of screw-pressed flaxseed oil. Choo et al. **[15]** have reported the physicochemical and quality characteristics of seven cold-pressed flaxseed oils sold in New Zealand. The characteristics of flaxseed hull oil were reported by Oomah and Sitter **[16]**.

Siccative vegetable oils have the property of oxidizing in the open air to form a hard, resistant and impermeable film, capable of protecting the substrate on which it has been applied in thin layers.

The first part of this manuscript will be devoted to the bibliographical study on the subject. We will see, first, some generalities on the vegetable oils. Then, the different possible chemical modification reactions on triglycerides will be reviewed. Finally, the principle and the basic notions of photopolymerization will be presented at the end of the chapter.

The second part of this thesis will consist in the characterization of linseed oil, as well as in the study of the chemical composition in fatty acids of this oil.

The experimental results will be reviewed during the results discussion.

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# Chapter I: Vegetable oils and triglycerides

# Chapter I: Vegetable oils and triglycerides

## I.1. General Information on vegetable oils:

### I.1.1 Treatment of oils:

Many steps are necessary to pass from vegetable seed to vegetable oils. **(Figure 1)** illustrates these different steps applied on Flaxseed.

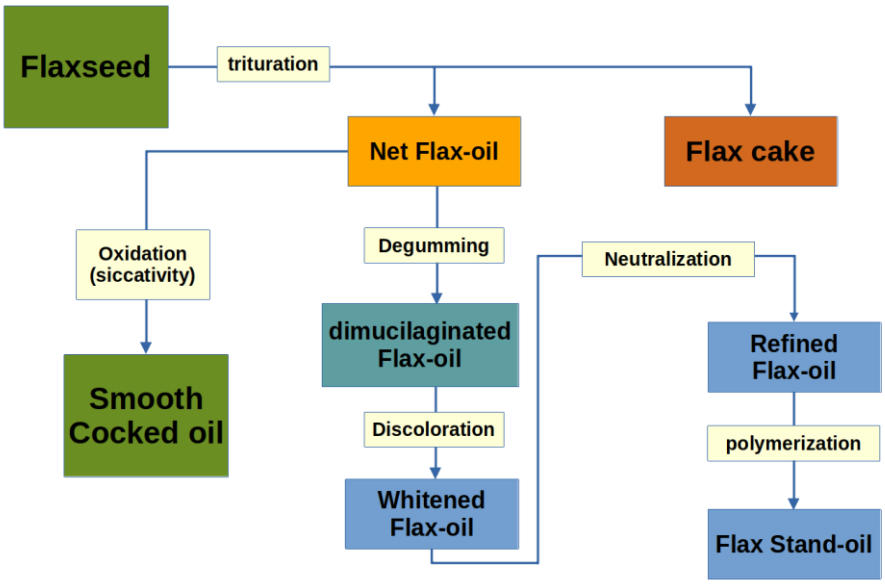



**Figure 1:** Flax-oil treatment.

Trituration is the operation that consists on extracting oil from seeds, we grind seeds by friction, and this operation is divided into four steps:

- Grinding.
- Mixing.
- Solid/liquid separation.
- Liquid/liquid separation.

At this process, Net oil is obtained alongside with Flax cake. The oxidation consists on **<<drying>>** the obtained oil due to the airs oxygen. Degumming permits to clear oils from elements that are not triglycerides. Gums are hydrolyzed in acid medium and then detached from triglycerides.

Discoloration serves eliminating pigments contained in greasy corps, yet in the price of structural collapse by time, back then we only change the color of the oil.

Neutralization serves eliminating fatty acids that are considered as impurities in refined oils and major suspects in accelerating oils oxidation, it's done by using caustic soda followed

by water washing, and finally drying. Polymerization permits thermal reticulation of fatty acids chains in oils, this operation is also called standolization.

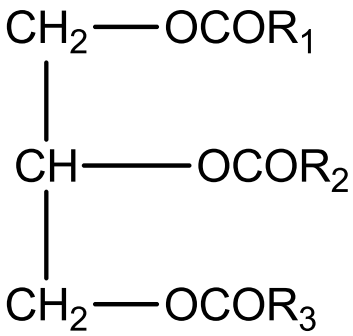
**I.1.2 Usage of oils:**

Only one third of Fats global production is directed to industrial usage. And by effect the remaining two thirds are directed to food production. And from the industrial usage of fats we mark the fabrication of soaps, fatty acids … etc

Triglycerides are the origin of numerous chemical products that enters in the composition of multitude products such as lubricant **[1]**-cosmetic products **[2]**-pharmaceutical products **[3]**-dyes and paints **[4]** …. Etc.

**I.1.3 Composition of oils:**

Vegetable and animal sourced oils are triesters made of glycerol ($HOCH_2$-CHOH-$CH_2OH$) and fatty acids (R-CO-OH), the general structure is as followed:




$R_1$, R2, R3 are long alkyled chains (in most of fatty acids, $C_{12}$, $C_{14}$, $C_{16}$ or $C_{18}$ saturated or not). Triglycerides are homogeneous, it means that $R_1 = R_2 = R_3$ or mix because $R_1$, R2, R3 are different. Many fatty acids enters in the composition of each oil and the triglycerides are the most found mixes.

The compositional difference of oils in fatty acids modifies the physico-chemical characteristics of oils. Yet the fusion temperature of a triglyceride is so variable in function of the double bond presence or not and the unsaturations isomerism of fatty acids **[5]**. in fatty acids presented in vegetable oils and at ambient temperature, the double bonds adopts **Z** configuration **(cis) [5]**. the official nomenclature "isomer cis/trans" <<Z/E>> however, in the case of unsaturated fatty acids, the confusion isn't possible to do in the absence of ramification and hetero-atoms, an isomer **cis** is always **Z** and an isomer **trans** is always **E**.

The compositions of the most common oils are given in **(Table 1)**, instead of absolute values we indicate forks of percentage given in the litterateur **[6-7]**. The fatty acids mass percentage in each type of oil varies according to many parameters, such as the climate and geographic origin, variety of the plant, harvest season ….etc. **[8]**.

**Table 1:** Average fatty acid compositions of certain vegetable oils**.**

| Oils | C18:3(%) | C18:2(%) | C18:1(%) | C18:0(%) | C16:0(%) |
|---|---|---|---|---|---|
| **Flaxseed oil** | 48-58 | 13-18 | 17-22 | 4-7 | 5-7 |
| **Soybean oil** | 6-12 | 46-56 | 20-30 | 2-7 | 7-10 |
| **safflower oil** | 1 | 62-80 | 10-20 | 2-7 | 6-8 |
| **Clove oil** | 0 | 65-80 | 20-25 | 2-5 | 4-8 |

## I.2. Characteristics of oils:

### I.2.1 Acid value:

The acid value testifies the <<Freshness>> of an oil and corresponds to the necessary mass of potassium hydroxide (KOH) to add for a gram of oil, at the end of neutralizing all the free fatty acids in test taking **[9-10]**. The Acid value is determined by an Acid/Base dosage in return. Fat corps reacts with a known excess of alcoholic Potash according to following reaction:

$$RCOOH + HO^- \longrightarrow RCOO^- + H_2O$$

The excess of potash is the dosed with a hydrochloric acid solution.

### I.2.2 Iodine value:

The three chains of fatty acids are not necessarily identical, the one that is in majority proportion gives the siccative characteristics to the oil. Thus, oils are classified into three categories: siccative, semi-siccative or non-siccative. These categories are defined according to either their iodine index **[11]**, or their drying index **[12]**.

The iodine value expresses the degree of establishment of a fat. It can be determined by measuring the double bonds by diiodine, and then corresponds to the mass of diiodine, expressed in grams, fixed per 100 g of fat. To facilitate the addition reaction, Wijs reagent (ICl) is preferentially used in $I_2$.

First, ICl is introduced in excess and reacts on the double bonds, then the excess ICl is transformed into $I_2$, and the diiodine formed is then dosed with potassium thiosulfate.

The three categories of oils and their respective iodine values are shown in **(Table 2)**.

**Table 2:** Classification of vegetable oils according to their iodine value.

| Oil type | Iodine value |
|---|---|
| Siccative | > 150 |
| Semi-siccative | 110 to 150 |
| Non siccative | 0 to 110 |

However, the iodine value must be completed by the position of the double bonds and their conjugation in order to correctly characterize the siccativity of an oil. Indeed, for a triglyceride of a given composition, the relative position of the fatty acids within the triglyceride modifies the chemical reactivity of the triglyceride **[13-14]**.

Linseed oil is the most commonly used siccative oil. The main fatty acid in this oil is linolenic acid, which contains three instaurations. It has an iodine number of 180.

The most common semi-siccative oil is soybean oil. Soybean oil has an iodine value of 130 and is composed mainly of triglycerides of linoleic and oleic acids.

Regarding non-siccative oils, we can cite castor oil composed mainly of ricinoleic acid, which is a monounsaturated fatty acid, hence the low iodine value of this oil (about 80-90).

**I.2.3 Ester value:**

The ester value (EV) of a fat is the number of milligrams of potassium hydroxide (KOH) required to neutralize the acids released by the hydrolysis of the esters contained in 1 g of fat. In particular, the ester value is equal to the saponification value for pure glycerides. In practice, this value is not measured experimentally, but is rather deduced by differentiating between the saponification value (SV) and the acid value (AV) **[94]**.

**I.2.4 Saponification value:**

The saponification number (SV) is defined as the number of milligrams of potassium hydroxide (KOH) required to saponify one gram of fat. This parameter has been determined according to the protocol described in the NF T 60-206 standard **[95]**, this procedure is based on reflux boiling of the sample with an ethanolic solution of potassium hydroxide, followed by titration of the excess potassium hydroxide with a titrated solution of hydrochloric acid. As for linseed oil, the (SV) is limited in the interval of 188-195 mg KOH per g oil **[96]**.

**I.2.5 Peroxide value:**

The peroxide value of a fat reflects the degree of its oxidation taking place. There usually are legal or quality limits for the (POV) .All substances that oxidize potassium iodide under the reaction conditions are determined by this method **[97]**. Based on treating the sample in solution with a mixture of acetic acid and a suitable organic solvent and then with a solution

of potassium iodide. The liberated iodine is titrated with a standard solution of sodium thiosulfate. Peroxide values are expressed either in milliequivalents of peroxide/kg or in millimoles of peroxide/L **[98]**.

Such as for linseed oil the recommended value is about 2 milliequivalents maximum of active oxygen per kg of oil **[96]**.

**I.2.6 Moisture (water and volatile content):**

It's the sudden loss of the sample mass after heating at 103 ± 2°C expressed in mass percentage, it consists on provoking water departure by heating a known quantity of oil until the complete elimination of water **[99]**, the recommended value for flaxseed oil is determined at 0.2% maximum **[96]**.

**I.2.7 pH:**

pH (potential hydrogen) is commonly used as a measure of the hydronium ion concentration in chemistry, biochemistry, soil science, wine science, and other fields:

$$pH = -\log_{10}[H_3O^+_{(aq)}]$$ **[100]**

it helps to determine the character of a solution either acid or alkaline in the interval of 0 to 14 according to Soren Sorensen (year of 1909).

**I.2.8 Refractive index:**

Refractive index is a basic value that relates to molecular weight, fatty acid, chain length, degree of unsaturation, and degree of conjugation. Refractive index is the degree of deflection of a beam of light that occurs when it passes from one transparent medium to another. A refracto-meter with temperature control is used for fats and oils with measurement usually at 25°C. A mathematical relationship between refractive index and iodine value was proposed by Zeleny, et al. **[101]**:

**Refractive Index at 25°C = 1.45765 + 0.0001164 × Iodine Value**

As for linseed the refractive index is recommended at 1.478 – 1.483 (Neutral Density 40°C) **[96]**.

Remark: increasing temperature effects the refractive index since there is a reciprocal relation between each other.

**I.2.9 Siccativity:**

The siccativity of vegetable oils is an indication of their ability to "dry" at room temperature in the presence of oxygen in the air. The siccativity is officially defined as "the property that certain substances applied in a thin layer possess, to evolve irreversibly from the liquid state

to the solid state by oxidative polymerization under the action of air, and possibly light" **[15]**. This property is due to the presence of instaurations contained in the aliphatic chains of the fatty acids that make up the oil.

In the presence of oxygen in the air, the double bonds induce radical polymerization and thus drying of the material.

The oxidation of the oils was highlighted by the absorption of oxygen molecules. Until 1950, two hypotheses concerning the reaction of oil with oxygen coexisted **[16]**. The first hypothesis assumed saturation of the ethylenic bond by oxygen fixation, while the second implied the formation of hydroperoxides on allylic carbon. This second hypothesis proved to be correct because it was observed that hydroperoxides were formed while the double bonds were preserved **[17]**. It appeared that the oxidation mechanism can only be explained by a radical reaction involving the removal of an allylic hydrogen atom by a free radical **[18]**.

Indeed, oils contain easily oxidizable impurities, such as hydroperoxides formed during the manufacturing process, etc. **[17]**. The radicals (noted $X^{\bullet}$) resulting from the thermal decomposition of these impurities, or generated in contact with the atmosphere, can tear a hydrogen atom from the fatty acid chain and initiate a radical oxidation reaction as shown in **(Figure 2) [19-20]**.

$$RH \xrightarrow{X^{\bullet}} R^{\bullet} + HX \qquad (1)$$

$$R^{\bullet} + O_{2(g)} \longrightarrow ROO^{\bullet} \qquad (2)$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \qquad (3)$$

**Figure 2:** Oxidation reaction by $O_2$.

In fact, the reaction (2) must be broken down into several steps. Indeed, the R- radical (in the case of methyl linoleate or any other fatty acid ester with a methylene group activated by double bonds) can evolve towards a more stable state where the double bonds are conjugated and whose configuration may have been modified. This radical isomerization reaction is very rapid and precedes the addition of oxygen to the radical. The formation of hydroperoxides can thus be interpreted by the mechanism **[21]** shown in **(Figure 3)**.

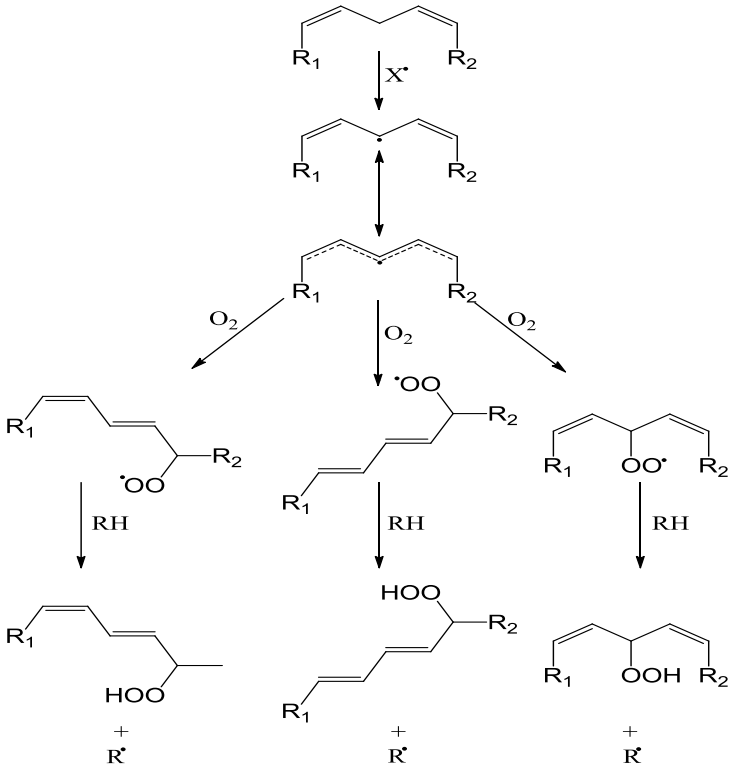



**Figure 3:** Hydroperoxides formation mechanism**.**

## I.3. Methods of oil analysis:

Advances in analytical methods may dispense with some of the chemical manipulations mentioned above.

For example, the iodine value can be determined by RAMAN spectroscopy **[22-23]** by measuring the intensity of the band (c=c) around 1650 $cm^{-1}$. The iodine value can also be determined by $^{1}H$ NMR **[24]**, as well as by FTIR spectroscopy **[25]** (Fourier transform infrared). The acid number can be obtained by FTIR spectroscopy **[26]** which allows to quantify the free acids contained in fats by measuring the intensity of the band (c=o) around 1710 $cm^{-1}$.

The techniques that provide the most information about a fat are undoubtedly the chromatographic methods. In particular, knowledge of the fatty acid composition of an oil often allows its identification. Most often this fatty acid composition is determined after transesterification of triglycerides into fatty acid methyl esters and separation of these methyl esters by gas chromatography **[27-28]**. The methyl esters are identified by the equivalent carbon number or equivalent chain length method **[29]**, which is related to the retention time. Retention times also vary according to the position of the double bond on the carbon chain **[30]**. The composition of triglycerides in oils can be obtained by coupling liquid

chromatography with a laser light scattering detector **[31]** as well as $^{1}H$ NMR **[32]**.Capillary electro-chromatography seems to improve the resolution compared to liquid chromatography **[33]** for the analysis of triglycerides in vegetable oils.

Finally, it should be noted that "trans" isomers can be differentiated from "cis" isomers according to their retention time in gas chromatography **[34]**.

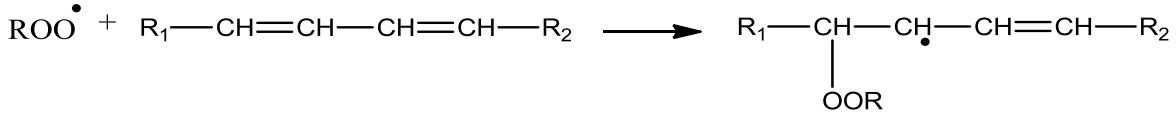
## I.4. Triglyceride reactions:

During the various studies aimed at understanding the reactions taking place between triglycerides, the authors found that the two major factors were temperature and oxygen. Indeed, by varying these parameters, the reactions on triglycerides are not similar. This is why we have distinguished two areas:

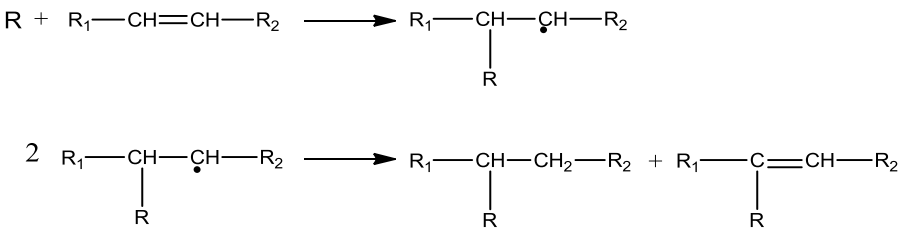
- At low temperature (between 60 and 100°C) and in the presence of oxygen.
- At high temperature (above 200°C) and in the absence of oxygen.

### I.4.1. At low temperature (between 60 and 100°C) and in the presence of oxygen:

The first mechanism proposed by Mayo **[35]**, implements an addition reaction of radicals directly on the conjugated double bonds. Mayo observed during alkene oxidations that the addition reaction of a peroxy radical on the double bond is very predominant for alkenes with conjugated double bonds or with allylic hydrogen atoms that are not very reactive.



Recently, Muizebelt **[36]** demonstrated the radical addition on the conjugated double bonds of ricinoic acid by mass spectrometry. The addition and dismutation reactions lead to different masses.



These reactions only occur for a fatty acid with conjugated double bonds (the radical formed is then stabilized by resonance) and are therefore not observed with linoleic acid. This mechanism of radical addition on the double bonds may constitute another possible way of cross-linking.

Higuchi et al **[37]** have transposed this mechanism to photo-oxidized safflower oil. The disappearance of the double bonds would be due to the addition of a peroxy radical ROO· on the conjugated double bonds and the authors observed by RAMAN spectrometry a band at 870 $cm^{-1}$ which they attribute to the presence of peroxides.

A second mechanism mentioned to explain the disappearance of double bonds is the formation of epoxides **[38]** or epidioxides **[39]**. However, the low concentrations of epidioxides obtained by the various authors do not allow these products to be considered responsible for the disappearance of double bonds. On the other hand, epoxides have often been identified either for low degrees of oxidation (dietary fats) **[18]** or for higher degrees of oxidation (case of paints) **[36]**. Epoxies can be formed by reaction of hydroperoxides on the double bonds and thus contribute to the disappearance of instaurations **[40]** (**Figure 4**).

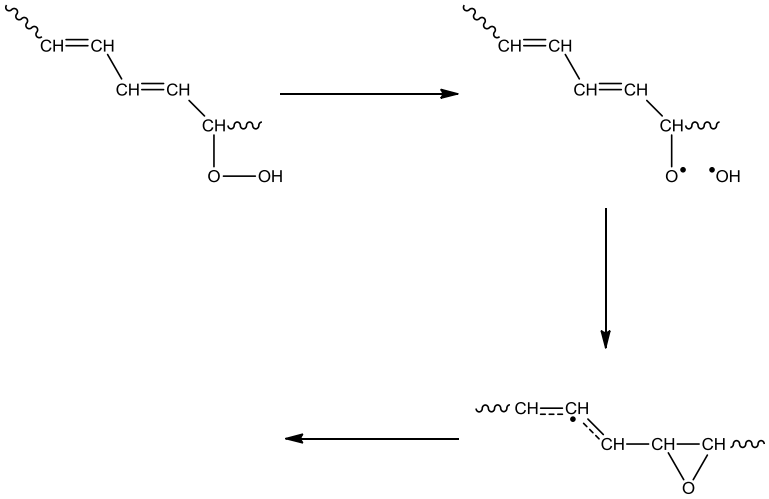



**Figure 4:** Epoxy formation by reaction of hydroperoxides on double bonds.

Two other routes of epoxide formation have been proposed **[41-42]**. The two possible reactions are shown in **Figure 5** and **Figure 6**. These reactions can also be used to justify the saturation of fatty acid chains.

The appearance of hydroperoxides has been observed by IR spectrometry with a wide absorption band in the hydroxyl zone around 3440 $cm^{-1}$ **[33]**. However, in order to detect low oxidation rates, it is necessary to use derivation methods that allow identification of oxidation products at much lower concentrations **[44]**. The formation of hydroperoxides has also been studied by UV-visible spectrometry **[45]** and chromatographic methods coupled with mass spectrometry.

**I.4.2. At high temperature (above 200°C) and in the absence of oxygen:**

The reaction involved is called standolization. It is a reaction that consists of heating raw oils to high temperatures. The "cooked oils" resulting from this reaction are called standolics,

after a Dutch name. During standolization, a thermal polymerization takes place which increases the viscosity of the oils and thus their speed of "drying", promoting the formation of a particularly flexible and impermeable protective film.

This reaction was the subject of numerous studies in the 1950s. Indeed, several theories **[46]** have been proposed to try to explain the standolization mechanism, but none of them allow us to assert it precisely.

The first theory developed in the 1930s by Kappelmeier **[47]** and Scheiber **[48]** defends the principle of the formation of covalent bonds between triglycerides, which can lead to gelation by the formation of a three-dimensional network. This hypothesis comprises two stages (**Figure 5**). First, isomerization into a system of conjugated double bonds, a relatively slow reaction imposing speed. Then, a rapid Diels-Alder reaction between the glycerides, leading to a progressive addition of the molecules.

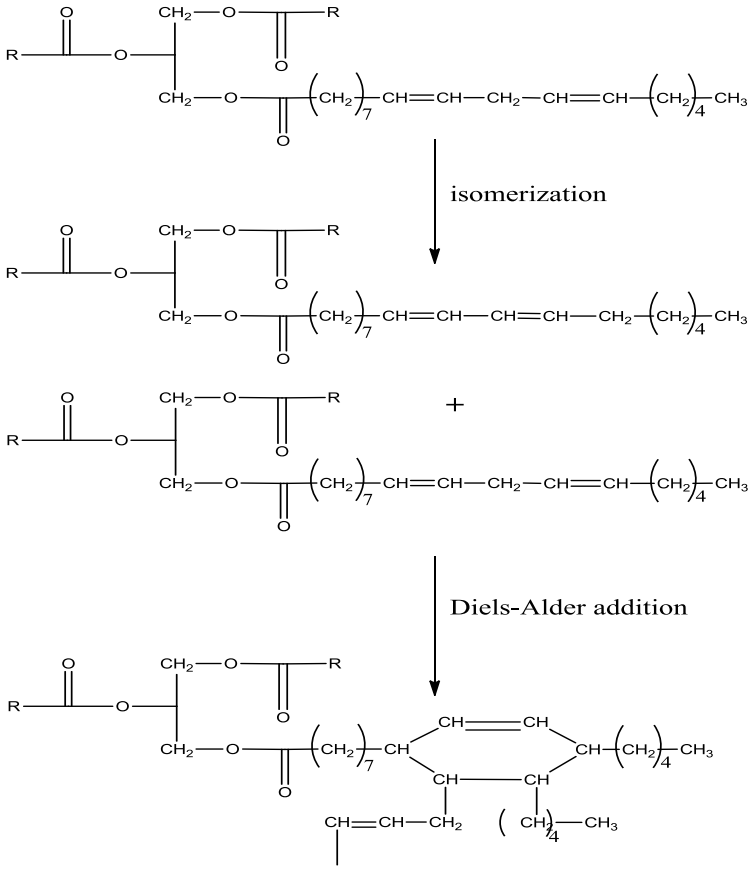



**Figure 5:** Kappelmeier-Scheiber Theory: Inter-glyceride Reaction.

In 1943, Pestemer and Tschinkel **[49]** as well as Mendl and Maschka **[50]** criticized this theory on the basis of other experimental observations. In fact, they observed a very rapid decrease in the number of unsaturations at the beginning of the reaction and a much slower increase in molar mass than could be expected. On the other hand, at the end of the reaction,

the number of unsaturations varies very little while the molar mass increases rapidly. Pestemer and Tschinkel therefore proposes a second theory that would explain these phenomena by a preliminary reaction between two unsaturated groups within a triglyceride molecule, a reaction referred to as the "intra-glyceride reaction". This reaction would not change the molar mass but would take place between radicals of acids esterified by the same glycerol molecule, resulting from an intradimerization. The final increase in molar mass would result from an intermolecular reaction between dimers and/or monomer groups (**Figure 6**).

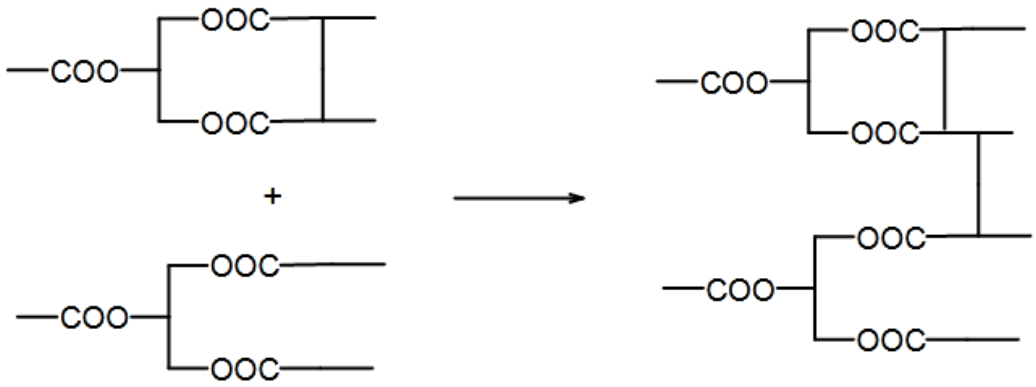



**Figure 6:** Reaction mechanism proposed by Pestemer and Tschinkel.

In 1958, Rushman and Simpson proposed a third theory called the theory of methylenic addition. They studied the polymerization kinetics of non-conjugated methyl linoleate, particularly at the beginning of the reaction. They then showed that dimerization has a finite initial rate, whereas the initial concentration of the conjugated isomer is zero.

Isomerization is therefore not a necessary condition for polymerization. The formation of the conjugated isomer occurs at the same time as dimerization. The proposed mechanism is then as follows:

1) Exchange of an methylenic hydrogen between two linoleate molecules with formation of free radicals (**Figure 7**).

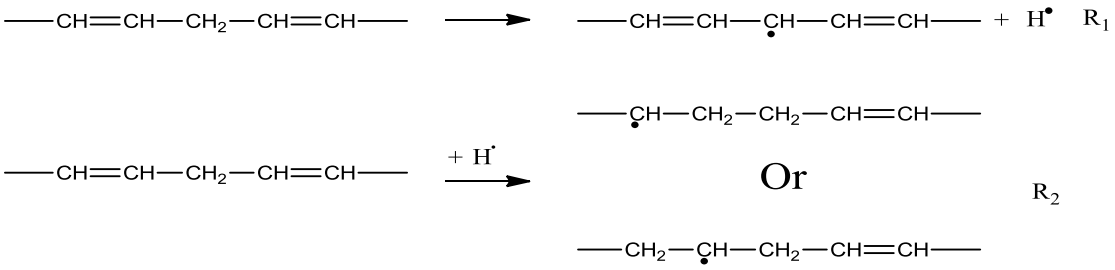



**Figure 7:** Mechanism proposed by Rushman et al.

2) $R_1$ is stabilized by resonance (**Figure 8**).

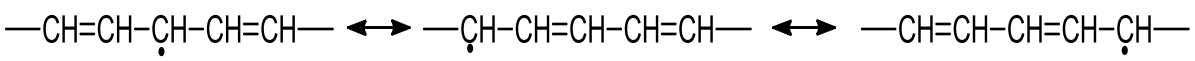


**Figure 8:** Mesomeric forms.

3) Dimers can be formed by reactions between $R_1$ and $R_2$, and then give non-cyclic compounds (**Figure 9**).

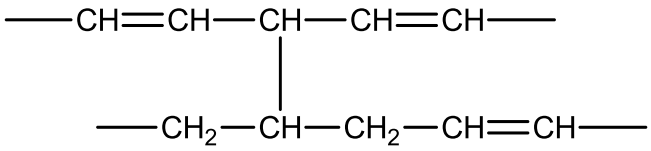


**Figure 9:** Formation of dimers.

However, Rushman and Simpson **[51-52]** recognize that there must be a subsequent cyclisation, the mechanism of which they do not indicate.

It has also been shown that at room temperature, the double bonds of triglycerides that are in cis-configuration change to trans-configuration when the oils are heated at high temperature. Hénon et al **[53]** have studied the isomerization of linoleic acid and linolenic acid and determine the degree of isomerization (DI). The degree of isomerization is the ratio between, for example, the proportion of trans linoleic acid and the total proportion of linoleic acid in the mixture. It has been shown that the degree of isomerization of linoleic acid, as well as that of linolenic acid, is independent of the initial composition of the oil, but varies according to the temperature and heating time. The transition from cis to trans configuration takes place above 210°C for linolenic acid and above 250°C for linoleic acid **[53]**.

Moreover, when oils are heated at high temperature for several hours, the amount of conjugated dienes increases up to a threshold viscosity value and then decreases rapidly, in direct relation to the rapid disappearance of non-conjugated linolenic acid **[54]**. This thus indicates that a polymerization reaction is taking place.

The global standolization scheme is certainly very complex, especially since it must be modified to take into account numerous secondary reactions whose respective importance remains to be evaluated. Addition reactions of the Diels-Alder and methylenic type are possible even if most authors favour the theory supported by Kappelmeier-Scheiber.

## I.5. Chemical modification of vegetable oils:

Vegetable oils all have several potentially reactive sites in their structure: unsaturations and ester functions. In addition to these two functional groupings, some vegetable oils have other chemical functions in their original state. For example, castor oil has hydroxyl functions on its fatty acid chains, and vernonia oil has oxirane rings.

We will review the different chemical modifications possible at the different reactive sites of vegetable oils.

### I.5.1. Reactions on unsaturations:

Triglyceride instaurations offer many possibilities to functionalize vegetable oils.

#### I.5.1.1. Epoxidation:

There are two main methods of epoxidation using either hydrogen peroxide (**Figure 10**) or peracids.

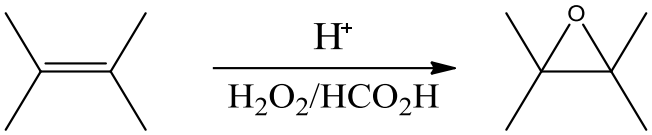



**Figure 10:** Epoxidation method for vegetable oils using hydrogen peroxide **[55]**.

The use of peracids is the most commonly used method in industry **[46-48]**. In this case, the epoxidation reaction takes place in solution (acetic acid) to avoid side reactions. In addition, this reaction requires good control of the operating conditions to obtain correct yields. Indeed, if the pH is too acidic or if the temperature exceeds 60°C, the epoxy cycles formed can react with the peracid and open into hydroxyl and acetoxy groups.

Epoxidation with hydrogen peroxide is less restrictive in terms of pH, temperature and use of solvents **[59-62]**. In addition, this epoxidation method does not give rise to side reactions. However, it requires the use of a phase transfer catalyst in order to obtain good yields **[63]**.

#### I.5.1.2. Styrenation:

Styrenation is a modification of vegetable oils that consists of grafting polystyrene molecules onto fatty acid chains. Styrenation makes it possible to insert 30 to 40% of styrene units in alkyd resins **[64]**.

Two styrenation methods are described: The "classical" method consists of creating radicals on allylic carbons through the thermal decomposition of a radical initiator such as benzoyl peroxide. Then, the homopolymerization of styrene makes it possible to obtain a polystyrene graft on the fatty acid chains **[65]** (**Figure 11**).

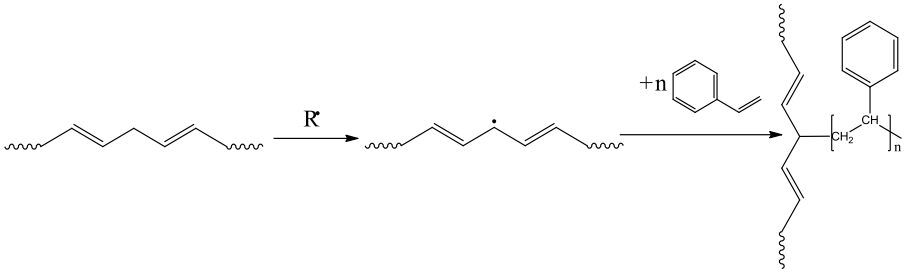



**Figure 11:** Vegetable oils "Classical" method of styrenation.

The second method, also known as the "macromonomer method", is carried out in several stages **[66]**. First, a transesterification takes place between vegetable oil and glycerol to obtain a mixture of glycerides with hydroxyl groups which are then esterified by the addition of acrylic acid. The thermal opening of the acrylic double bond then initiates the polymerization of styrene (**Figure 12**).

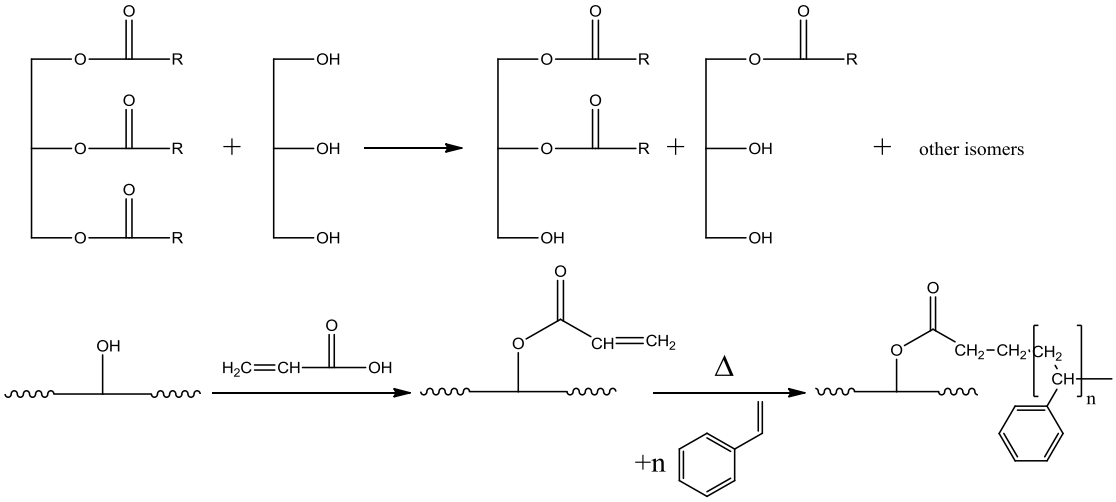
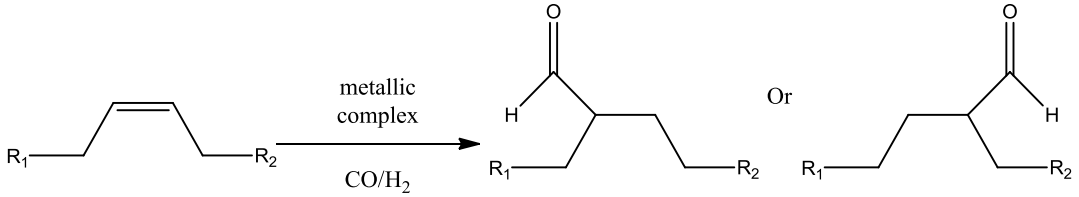



**Figure 12:** Macromonomer method for styrenation of vegetable oils.

### I.5.1.3. Carbonylation:

There are different methods of introducing a carbonyl group into fatty acid chains.

Hydroformylation **[67]**, also called oxo-process, allows the introduction of an aldehyde function on the triglycerides (**Figure 13**) using a metal complex, generally based on cobalt.

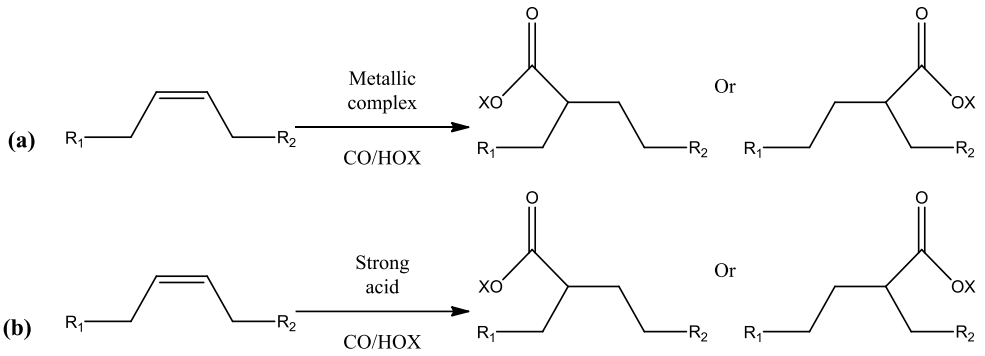



$R_1$ and $R_2$ = Alkyl, H or -COOR

**Figure 13:** Hydroformylation reaction on vegetable oil unstaurations.

The introduction of an aldehyde function on fatty acid chains offers a new synthesis route for polyols, polyacids as well as polyesters and polyamines. The products obtained are mainly used as plasticizers, in lubricants or paints **[68]**.

It is also possible to introduce carboxylic acid functions on the double bonds of oils. To do this, the two main reactions are hydroxycarboxylation and the Koch reaction (**Figure 14**).




**$R_1$ and $R_2$ = Alkyl, H or -COOR**
**X = H, Alkyl**

**Figure 14:** Hydroxycarboxylation reaction (a) and Koch reaction (b) for the introduction of a carboxylic acid function on the double bonds of vegetable oils **[69]**.

The introduction of a carboxylic acid function is interesting for the production of polyesters or polyamines.

### I.5.1.4. Selective hydrogenation:

The hydrogenation of oils consists in reducing the number of double bonds in order to increase their stability and especially to reduce their melting point **[70-71]** It is, however, not possible to obtain fully saturated oils **[72]** (**Figure 15**). Products resulting from hydrogenation are widely used as lubricants.

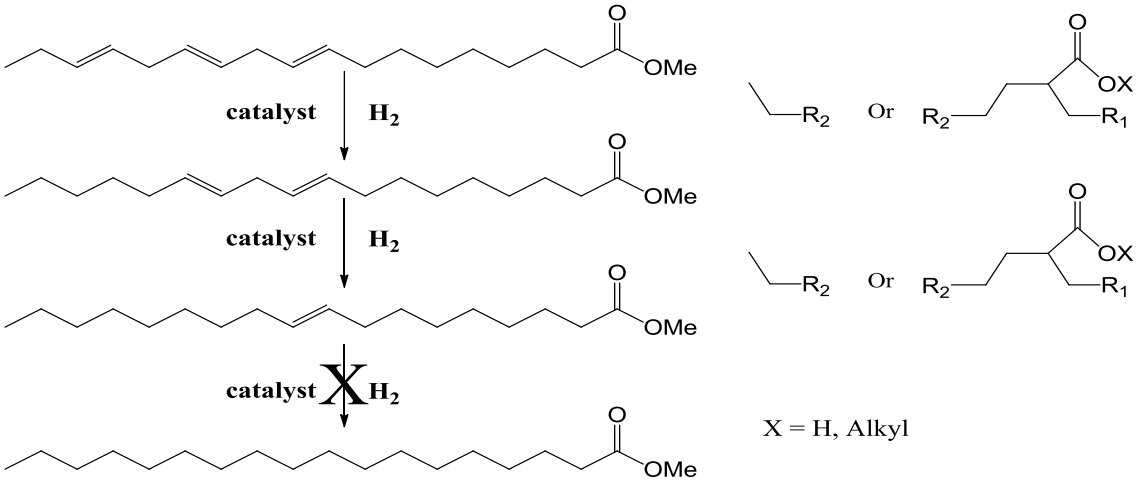



**Figure 15:** Selective hydrogenation of vegetable oils.

The main catalysts used are palladium or Raney nickel or metal oxides, such as copper-chromium oxide.

**I.5.1.5. Hydroxylation:**

The presence of hydroxyl groups on the fatty acid chains of oils makes many chemical modifications possible.

It should be noted that some vegetable oils, such as castor oil, already have hydroxyl functions in their original state. For oils without these functional groups, it is possible to introduce them by oxidation of the double bonds of the triglycerides (**Figure 16**), the so-called hydroxylation reaction **[73-74]**.

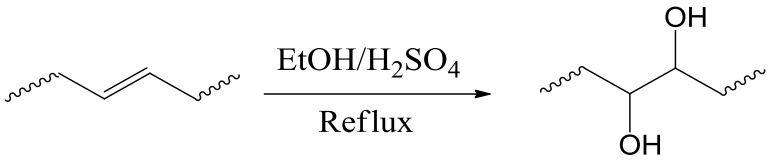



**Figure 16:** Hydroxylation of vegetable oils.

Hydroxylated oils can then act as polyols in the manufacture of polyurethane foams.

**I.5.1.6. Hydroxybromination:**

Apart from the unstaurations on the fatty acid chains of vegetable oils, these do not have any real chemical functions. Indeed, the modification of vegetable oils is not always easy on the double bonds. This is why certain reactions, such as hydroxybromination **[65]**, make it possible to introduce an anchoring function on the triglycerides as shown in **figure 17**.

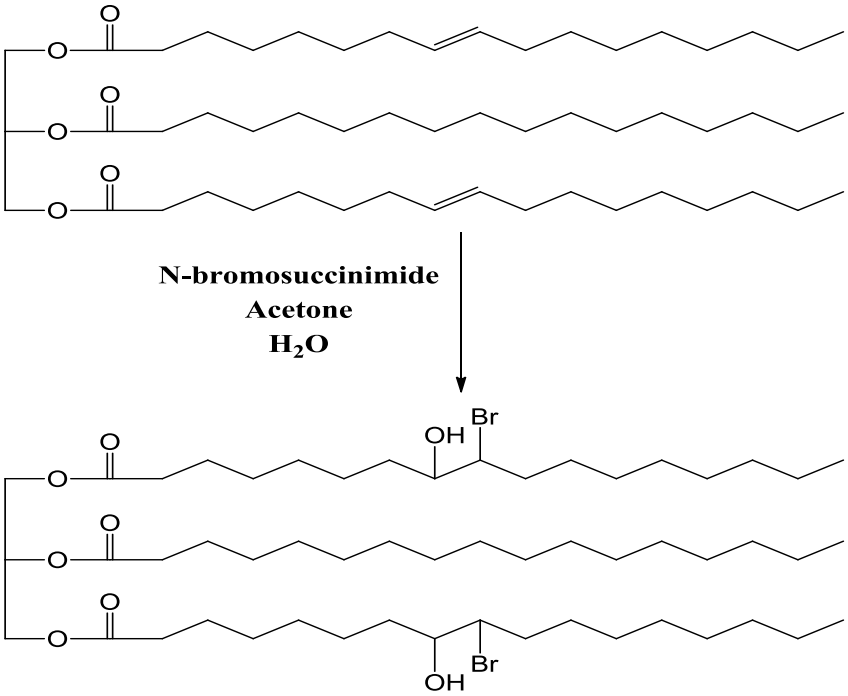



**Figure 17:** Hydroxybromination of triglycerides.

The interest of this reaction is to introduce two chemical functions, a hydroxyl function and a bromine, in a single step.

Generally, the products obtained during hydroxybromination are then used as copolymers or are modified by an acrylic function in order to be used for photopolymerization.

**I.5.1.7. Alkylation:**

Friedel-Crafts alkylation allows the introduction of an isopropyl group on the double bonds of the oils **[75-76]** (**Figure 18**). In this way, the oils become fully saturated, thus increasing their resistance to oxidation.

However, this process is not economical because it is necessary to use two molecules of ethylaluminum chloride for one molecule of fatty acid.

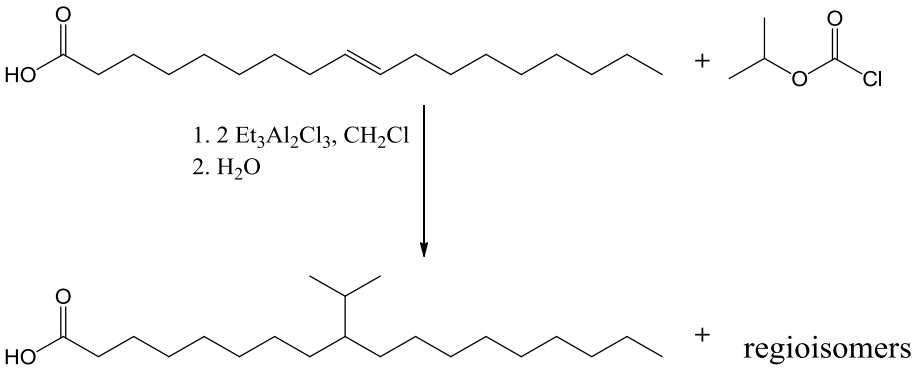



**Figure 18:** Friedel and Crafts alkylation reaction.

Oils modified by this reaction are used in the cosmetics industry **[72]**.

**I.5.1.8. Acylation:**

It is possible to introduce a ketone function on fatty acid chains using the Friedel-Crafts acylation reaction **[77-78]** (**Figure 19**).

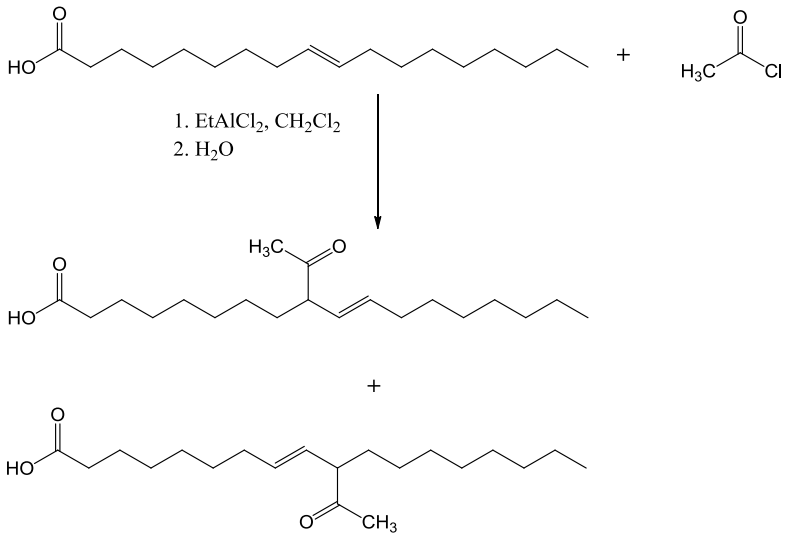



**Figure 19:** Friedel and Crafts Acylation Reaction.

Like the alkylation reaction, Friedel-Crafts acylation is not economical because it requires a large amount of very expensive catalyst.

**I.5.1.9. Maleinization:**

Another possible method to functionalize vegetable oils is maleinization **[79-82]**. This reaction consists in introducing anhydride functions on the fatty acid chains of the oils (**Figure 20**).

The major applications of maleinized oils are emulsifiers, cross-linking agents, etc. **[79-82]**.

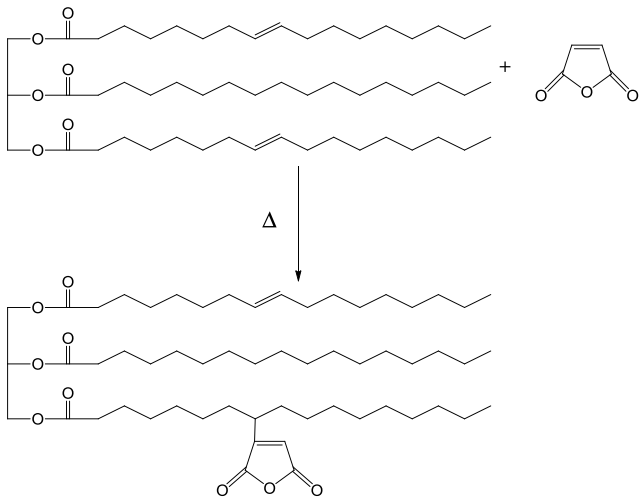



**Figure 20:** Maleinization of vegetable oils.

**I.5.1.10. Dimerization:**

There are two ways to dimerize the fatty acid chains of oils **[69]**, either by Diels-Alder-type addition (**Figure 21**) or by radical addition (**Figure 22**).

Diels-Alder addition dimerization involves, at least, a di-unsaturated acid that plays the role of diene.

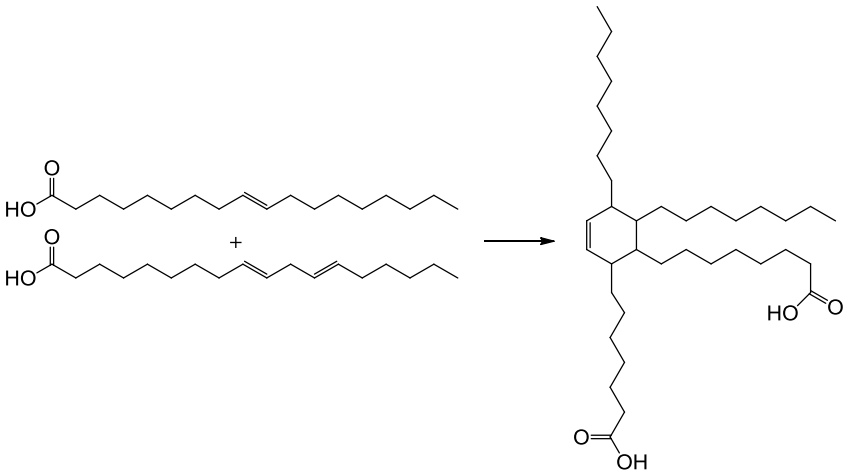



**Figure 21:** Dimer formation by Diels-Alder addition reaction.

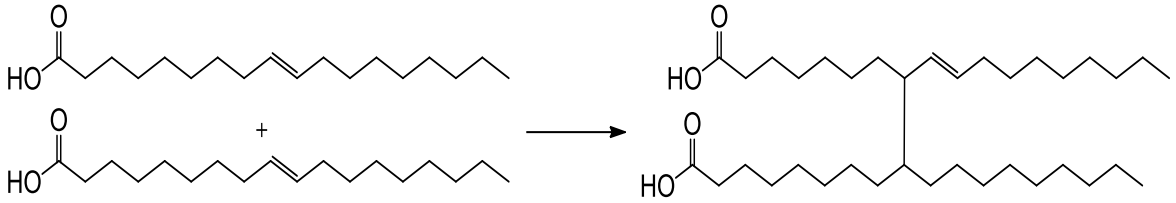


**Figure 22:** Formation of dimers by free-radical addition reaction.

The dimers obtained are, for example, used in the synthesis of polyesters or polyamides which will then be used in the elaboration of "hot melt" adhesives.

**I.5.1.11. Metathesis:**

The metathesis reaction **[83]** was discovered in the 1950s. The aim of this reaction is to form a new ethylenic compound from two ethylenic compounds (**Figure 23**).

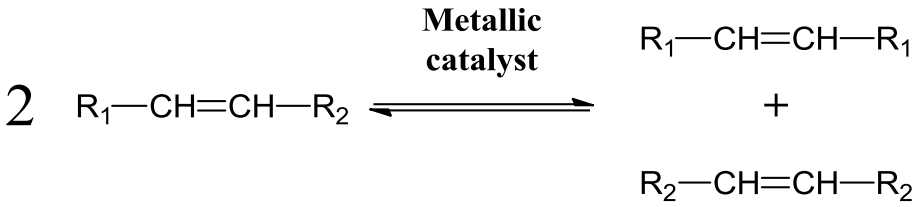



**Figure 23:** Principle of metathesis.

**I.5.2. Reactions on the ester functions:**

**I.5.2.1. Transesterification:**

The transesterification **[84-85]** of triglycerides is a reaction most often used to form mono or di-glycerides (**Figure 24**), either in the presence of a conventional catalyst ($H_2SO_4$, APTS...) or by an enzyme.

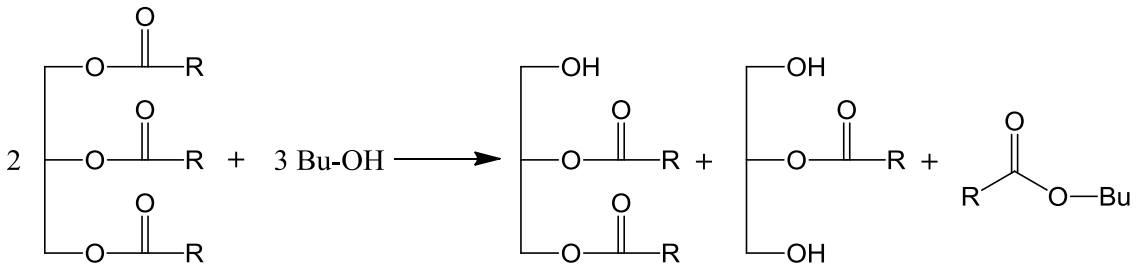



**Figure 24:** Transesterification of triglycerides.

A special case of alcoholysis (**Figure 25**) is glycerolysis **[59]**.

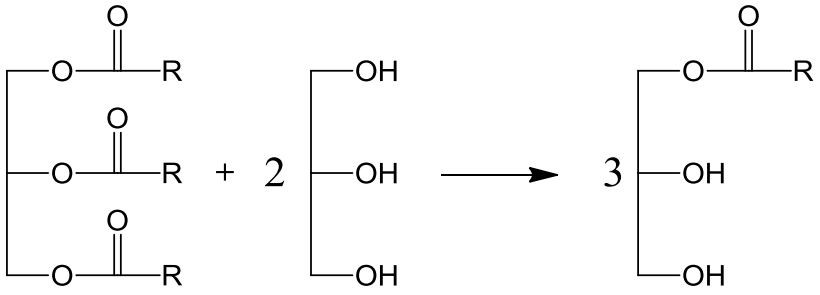



**Figure 25:** Glycerolysis reaction of vegetable oils.

**I.5.2.2. Hydrolysis:**

The hydrolysis **[69]** of triglycerides allows the fatty acids to be dissociated from glycerol (**Figure 26**).

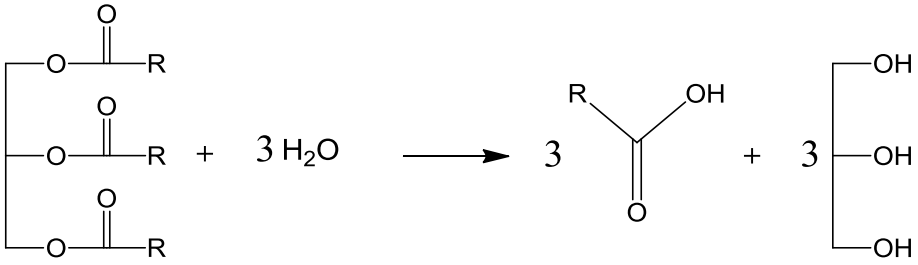



**Figure 26:** Hydrolysis of triglycerides.

This hydrolysis can be carried out with a catalyst such as an acid, a base or in the presence of an enzyme.

The fatty acids can then be used separately in different reactions.

**I.5.2.3. Amidation:**

Amidation **[86-88]** of triglycerides forms monoglycerides with one or two amine functions at one end (**Figure 27**).

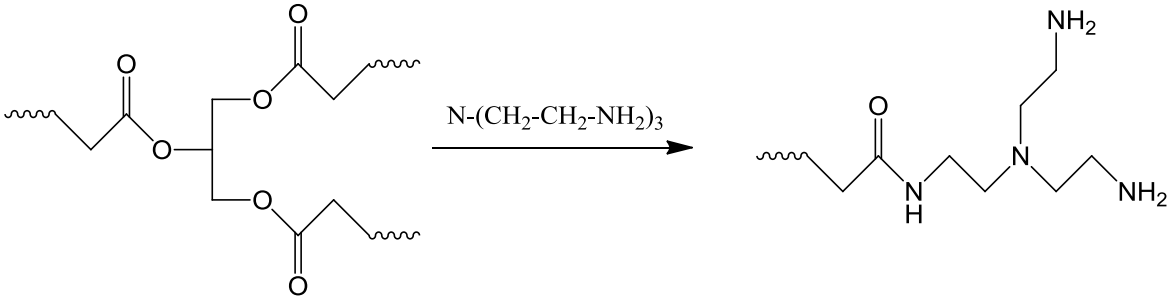



**Figure 27:** Monoglyceride formation by triglyceride amidation**.**

These compounds can be used as a reactive diluent or in alkyd resins.

## I.5.3. Reactions on hydroxyl functions:

The hydroxyl functions can be naturally present in oils (castor oil) or result from a first stage of modification.

The presence of hydroxyl functions on the fatty acid chains of oils then makes possible many types of chemical reactions.

**I.5.3.1. Dehydration:**

Dehydration **[89-90]** mainly concerns castor oil and is widely used industrially to produce drying oils often for paints and coatings.

Dehydration is carried out at high temperature (250°C) in the presence of a catalyst such as sodium bisulfate (**Figure 28**).

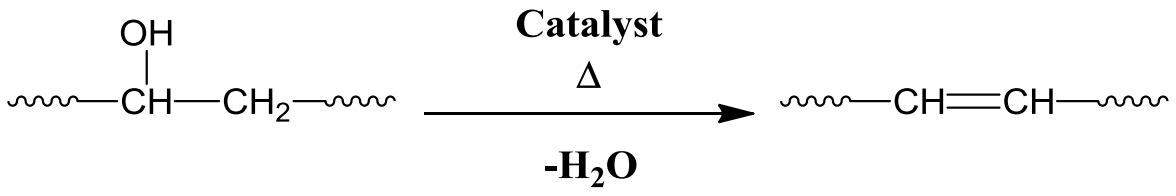



**Figure 28:** Triglyceride Dehydration.

Thanks to this treatment, the number of instaurations increases and, as a result, the oxidation-polymerization or siccativation power of the oils increases.

**I.5.3.2. Acetoacetylation:**

Acetoacetylation allows the introduction of ketones into the fatty acid chains of oils **[91]**.

To do this, hydroxyl groups react with t-butyl acetoacetate (**Figure 29**).

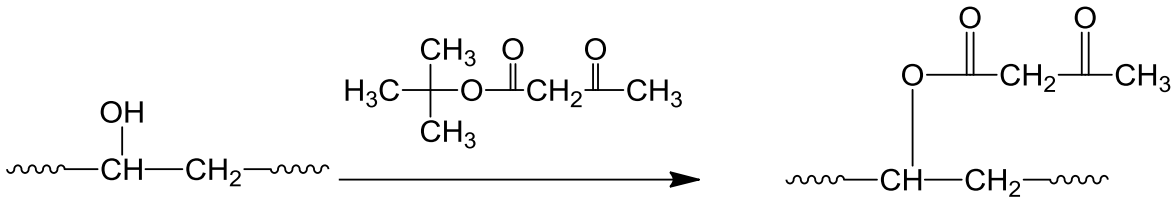



**Figure 29:** Acetoacetylation of Castor Oil.

The products obtained after acetoacetylation are for example, used in the formulation of coatings.

**I.5.3.3. Esterification:**

This reaction has been widely studied and can take place in both basic and acidic media (**Figure 30**) **[91-92]**.

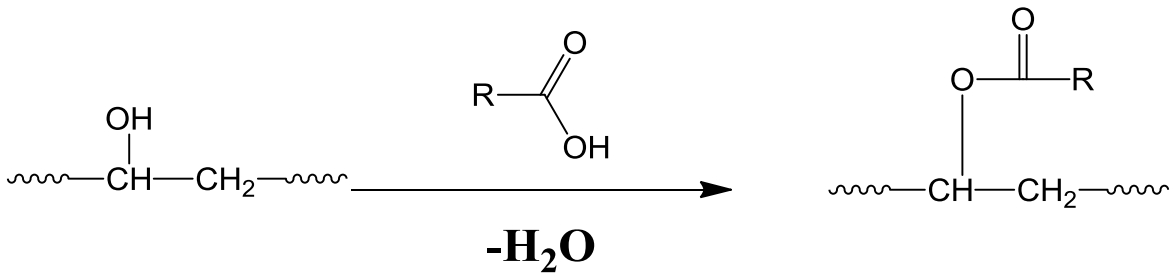



**Figure 30:** Esterification of oils from hydroxyl functions of fatty acid chains.

Fatty acid esters can be used as emulsifiers in food, as surfactants for soaps, as dispersing agents or in biological fuels.

Grafting of maleic anhydride can also take place on the hydroxyl functions of fatty acid chains **[93]** via esterification (**Figure 31**).

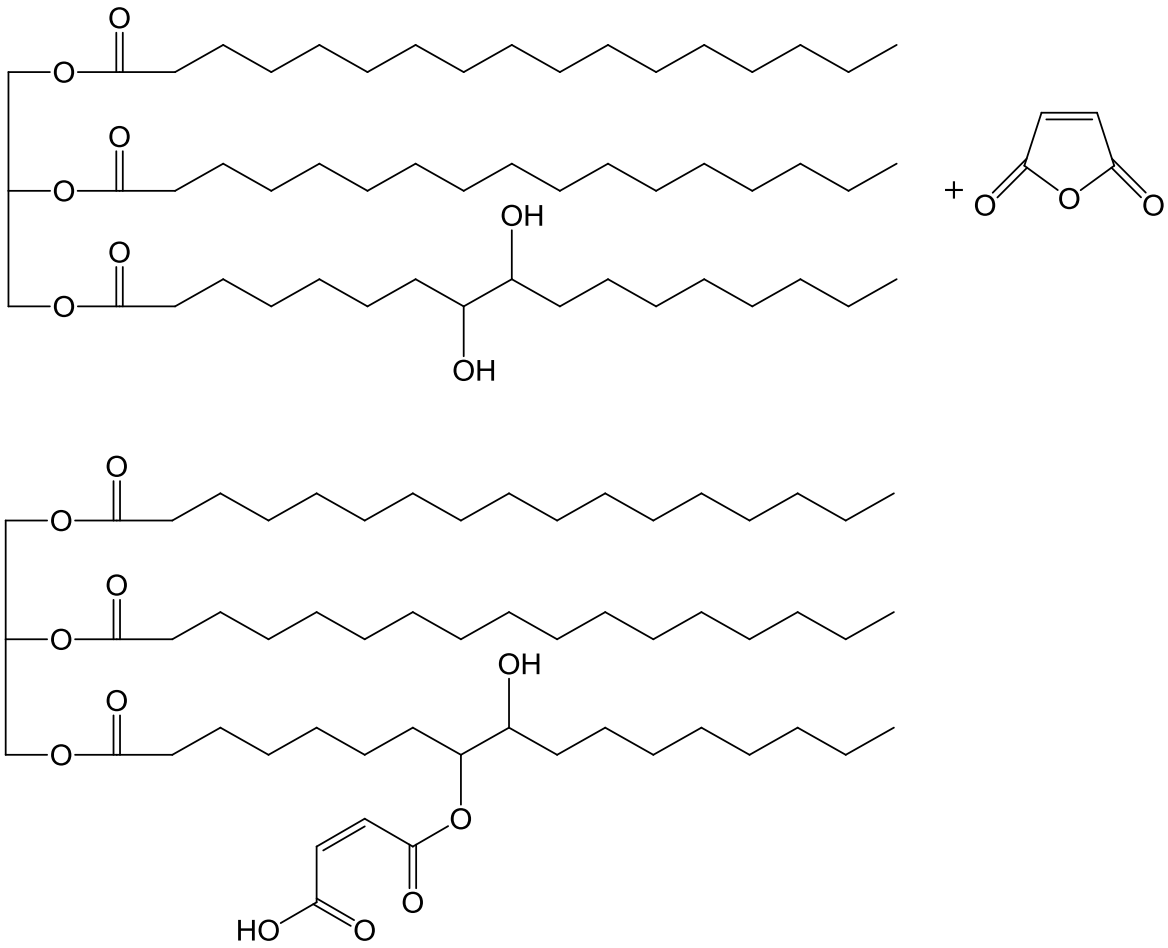



**Figure 31:** Esterification of hydroxylated oils with maleic anhydride.

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# Chapter II:
# Flaxseed oil

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## II.1. Flax:

### II.1.1. Definition:

Flax (Linum usitassimum) belonging to family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste **[1-2]**. The term "Flaxseed" is often used to describe flax when consumed by humans while linseed denotes when it is used specifically for industrial applications **[1]**, almost all parts of linseed plant are utilized for various purposes. Seeds contains oil, which after refining is used for edible purpose **[3-4]**.

### II.1.2. Cultivation of flax:

The cultivation of flax is one of the oldest utilitarian cultures, the first traces of its use date back to 8000 BC in Turkey **[5]**. Currently, flax is used in the textile (fiber), food (seed and oil) and chemical (oil) industries **[6]**

According to the botanical classification of Linnaeus, flax (Linum usitatissimum L ), an annual herbaceous species, belongs to the sub-branch of the Angiosperms, and is part of the order Linales, family Linaceae.

The uses of flax have led to the selection of two families of flax: fibre flax and oilseed flax. The main phenotypic difference between the two families lies in the plant's habit. Unlike fibre flax, oilseed flax has a higher rate of stem branching, which leads to higher seed production.

Commercial oilseed flax varieties are differentiated into spring and winter varieties, for a plant production adapted to climatic conditions (29 spring flax varieties versus 9 winter flax varieties are registered in the French catalog of 2012; GEVES, 2012). The winter lines are derived from the crossing of spring lines, and selected for their tolerance to low temperatures. Winter flax is sown in September and spring flax in March. Winter flax has a more stable seed yield, as the variety is more tolerant to cold and less sensitive to environmental stresses [7].

The evolution of the flax plant from the flower bud to the mature capsule is shown in **figure1** (photos 2 to 5). The vegetative period, allowing for the growth of stems and leaves (photo 1), extends from 45 to 60 days. During the following 15 to 25 days, flower buds appear at the end of the stems (photos 2 and 3). The flowers evolve into capsules during the maturation process (photo 4), which takes place over the next 30 to 40 days. At the end of the maturation, the plant is dry and yellowed (photo 5), the capsules containing the seeds are harvested. Each capsule has five chambers, containing a maximum of 10 seeds.


**Figure 1**: Flax cultivation in the field (photo 1) and evolution of the flax plant in the greenhouse, from the flower bud to the mature capsule (photos 2 to 5).

### II.1.3. Flaxseed composition:

Flaxseed holds a remarkable compositional diversity for being good source of alpha-linolenic acid, lignans, high quality protein, soluble fiber and phenolic compounds [8]. The nutritional composition of flaxseed is presented in **Table 1[1-9-10]**.

**Table 1**: Nutritional composition of flaxseed.

| Nutrients | Amount per 100 g of edible flaxseed |
|---|---|
| **Moisture (g)** | 6.5 |
| **Protein (N×6.25) (g)** | 20.3 |
| **Fat (g)** | 37.1 |
| **Minerals (g)** | 2.4 |
| **Crude fiber (g)** | 4.8 |
| **Total dietary fiber (g)** | 24.5 |
| **Carbohydrates (g)** | 28.9 |
| **Energy (kcal)** | 530.0 |
| **Potassium** | 750.0 |
| **Calcium (mg)** | 170.0 |
| **Phosphorous (mg)** | 370.0 |
| **Iron (mg)** | 2.7 |
| **Vitamin A (µg)** | 30.0 |
| **Vitamin E (mg)** | 0.6 |
| **Thiamine (B1) (mg)** | 0.23 |
| **Riboflavin (B2) (mg)** | 0.07 |
| **Niacin (mg)** | 1.0 |
| **Pyridoxine (mg)** | 0.61 |
| **Pantothenic acid** | 0.57 |
| **Biotin (µg)** | 0.6 |
| **Folic acid (µg)** | 112 |

Chemical composition of flaxseed depends upon growing environment, genetics and processing conditions **[1]**. The lipid content of flaxseed varies from 37 to 45 g/100 g of the seed as reported by various scientists **[1-10-11]**. Cotyledons are the major oil storage tissues, containing 75 % of the seed oil **[2-3-4]**. Flaxseed oil constitutes 98% triacylglycerol, phospholipids and 0.1%free fatty acids **[12]**. On an average, it contains 21 % protein. Majority of the protein is concentrated in the cotyledons **[13]**. Major protein fractions are globulin (26–58 %) and albumin (20–42 %). Nutritional value and amino acid profile of flaxseeds are comparable to that of soya proteins **[14-15]**.Flaxseed protein is rich in arginine, aspartic acid and glutamic acid, while lysine is limiting **[3-4-16]**. High cysteine and methionine contents improve the anti-oxidant levels, thus helps in reducing risk of cancer [8]. The processing conditions, dehusking and defatting affect the protein content. The defatted and dehusked meals have high protein content **[17-18]** Flaxseed proteins exhibit antifungal properties against Alternaria solani, Candida albicans and Aspergillus flavus **[19-20]**. Flaxseed is the richest source of phytoestrogens (lignans). The amount of secoisol ariciresinoldiglycoside (SDG) varies from 77 to 209 mg SDG/tbsp. of whole flaxseed **[1-21]**. Flaxseed contains very low level of carbohydrates (1 g/100 g) and thus contributing very little to total carbohydrates intake **[1]**. Flaxseeds contain a good amount of phenolic compounds. The sephenolic compounds are well known for anti-cancer and anti-oxidative

properties. Flaxseeds have three different types of phenolic compounds–phenolic acids, flavonoids and lignans. Major phenolic acids present in defatted flaxseed are ferulic acid (10.9 mg/g), chlorogenic acid (7.5 mg/g), gallic acid (2.8 mg/g). Other phenolic acids include p-coumaric acid glucosides, hydroxycinnamic acid glucosides and 4-hydroxybenzoic acid that are present in low quantities **[22-23]**. Flavone C- and Flavone O-glycosides are the major flavonoids found in flaxseeds **[2]**. It serves as a good source of minerals especially, phosphorous (650 mg/100 g), magnesium (350–431 mg/100 g), calcium (236–250 mg/100 g) and has very low amount of sodium (27 mg/100 g) **[1]**. It contains highest amount of potassium 5600–9200 mg/kg among various foods and high potassium intake is inversely related to blood platelet aggregation, free radicals in blood and stroke incidence **[11]**. Flaxseed contains small amounts of water-soluble and fat-soluble vitamins. Vitamin E is present as γ- tocopherol, amounting to 39.5 mg/100 g. γ-tocopherol is an antioxidant providing protection to cell proteins and fat from oxidation; promotes sodium excretion in urine, which may help in lowering of blood pressure and heart disease risks and Alzheimer disease **[1-24]**.

**II.1.3.1. Alpha-linolenic acid:**

Alpha-linolenic acid is the main functional component of flaxseed. It serves as an exclusive source of omega-3 fatty acid in the vegetarian diets **[25]**. Flaxseed oil is rich in polyunsaturated fatty acid (73 % of total fatty acid), moderate in monounsaturated fat (18 %) and low amount of saturated fat (9%) **[26-27]**. It is rich in both the essential fatty acids—alpha-linolenic acid (ALA), and linolenic acid (LA).

**Table 2** represents several reported Fatty acid profiles of various oilseeds. It is evident from the data that flaxseed contains highest amount of linolenic acid followed by soybeans and mustard oil, while sunflower and safflower oils contain large amount of linoleic acid, which may leads to various diseases. Over the past 100 to 150 years, the consumption of vegetable oils from corn, sunflower seeds, safflower seeds, cottonseeds and soybeans has greatly increased, which resulted in drastic imbalance of the essential fatty acids. Today, the ratio of omega-6 to omega-3 fatty acid is shifted to 20–30:1 in western diets and the situation is even worse in case of Indian diets where this ratio attains a high value of 38–50:1 which reveals that more of omega-6 fatty acids are incorporated into the cell membrane **[28-29]**. Therefore, the cellular functions support more of the pro-inflammatory processes than anti-inflammatory processes. Simple dietary choices, which favour foods containing omega-3 fatty acids, can ameliorate this imbalance. The recommended ratio of omega-6 to omega-3

fatty acids may be in the range of 4:1 to 10:1, and omega-6 and omega-3 fatty acid intakes should account for at least 3 and 0.5 % of total energy intake, respectively **[30-31-32]**.

**Table 2:** Fatty acid profile of various oilseeds **[27]**.

| Fatty acid | Flaxseed | Mustard | Soya bean | Rice bran | Corn | Sesame | Safflower | Olive | Sun flower |
|---|---|---|---|---|---|---|---|---|---|
| **Saturated** | 10 | 8 | 15.7 | 21.3 | 14.8 | 15.7 | 9.1 | 15.3 | 12.8 |
| **Mono unsaturated** | 18.5 | 62.4 | 24.2 | 42.4 | 28.1 | 40.1 | 13.9 | 73.8 | 22.4 |
| **Poly unsaturated** | 71.8 | 31.5 | 59.8 | 35.9 | 57.1 | 45.7 | 77.3 | 10 | 66 |
| **Linoleic acid (n6)** | 16.8 | 21.6 | 52.1 | 34.6 | 56.1 | 45.3 | 76.5 | 9.4 | 65.6 |
| **Linolenic acid (n3)** | 55 | 9.9 | 7.8 | 1.2 | 1 | 0.4 | 0.8 | 0.6 | 0.5 |
| **n6/n3** | 0.3 | 2.2 | 6.7 | 2 | 56 | 113 | 7.4 | 16 | 131 |

### II.1.3.2. Lignans:

Lignans are phytoestrogens, which are abundantly available in fiber rich plants, cereals (wheat, barley, and oats), legumes (bean, lentil, and soybean), vegetables (broccoli, garlic, asparagus, carrots) fruits, berries, tea and alcoholic beverages. Flaxseed contains about 75–800 times more lignans than cereal grains, legumes, fruits and vegetables **[33-34-35-36]**. Secoisolaricires in oldiglycoside (SDG) is the major lignan of flaxseed, along with minor contents of matairesinol, pinoresinol, lariciresinol and isolariciresinol **[37-38-39]**. SDG ranges from11.7to24.1mg/g in defatted flour and6.1to13.3mg/g in whole flaxseed flour **[40]**. Lignans are the diphenolic compounds synthesized by the coupling of two coniferyl alcohol residues existing in cell wall of higher plants **[21-41]**. Secoisolariciresinol (SECO) is produced by acid hydrolysis of secoisolariciresinol diglycoside. Secoisolariciresinol diglycoside existing bound form as a complex of five secoisolariciresinol diglycoside residues held together by four HMGA (3-hydroxy-3-methylglutaric acid) residues in the outer layers of the seed **[42-43]**. Structures of the flaxseed lignans complied from different sources **[21-37]** are presented in **Figure 2**.

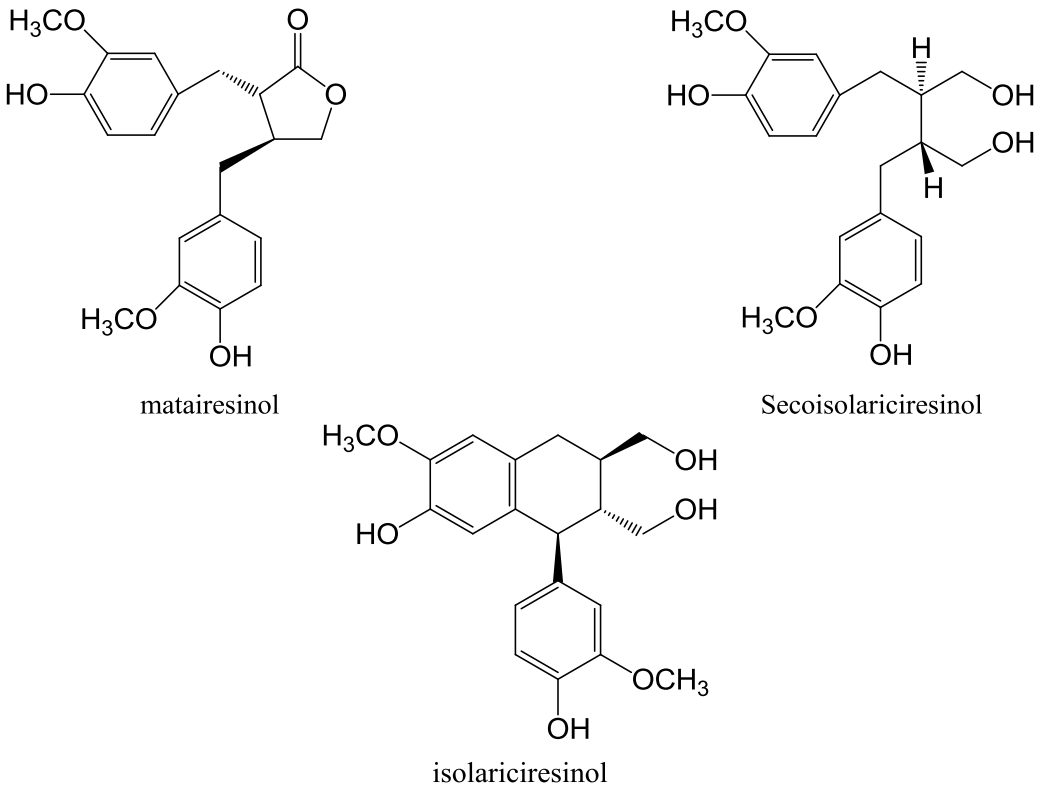


**Figure 2**: Structure of various lignans present in flaxseed

**II.1.3.3. Dietary fiber:**

Flaxseeds serve as a good source of both soluble and insoluble dietary fiber. Flaxseed holds a unique place among the oilseeds due to presence of mucilage located in outer layers of the seed **[3-4]**. Flaxseed mucilage has gained momentum due to its superb health benefits and potential functional properties **[44-45]**. It contains 35–45% of fiber and two third is insoluble and one third is soluble fiber. Insoluble fiber consists of cellulose, hemicellulose and lignin **[1-15]**. Most of the soluble fiber of flaxseed appears to be the mucilage of seed coat. It makes up 7–10 % of seed weight **[45]**. Soluble fiber in the form of mucilaginous material consists mainly of water-soluble polysaccharides; its recovery and purity vary with the extraction conditions. The water binding capacity of flaxseed mucilage is reported to be about 1600–3000g of water/100 g of solids. High water binding capacity of flaxseed is attributed due to the presence of polysaccharides in the seed coat **[46-47]**.

Mucilage of flaxseed consists of acidic and neutral polysaccharides. The neutral fraction constitutes L-arabinose, D-xylose and D-galactose and arabinoxylan and acidic fraction contains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid **[47]**. Functionally, these polysaccharides possess similar properties to guar gum **[48-49]**. The mucilage can be extracted by water and exhibit good foam-stability properties **[44]**.

## II.2. Flaxseed oil:

### II.2.1 Processing:

Commercial processing of flaxseeds is carried out to obtain oil and various by-products. Compositional changes during processing are of prime importance to food, feed and nutraceuticals industry. Processing of flaxseeds at commercial level involves multiple steps as shown in **Figure 3 [18]**.

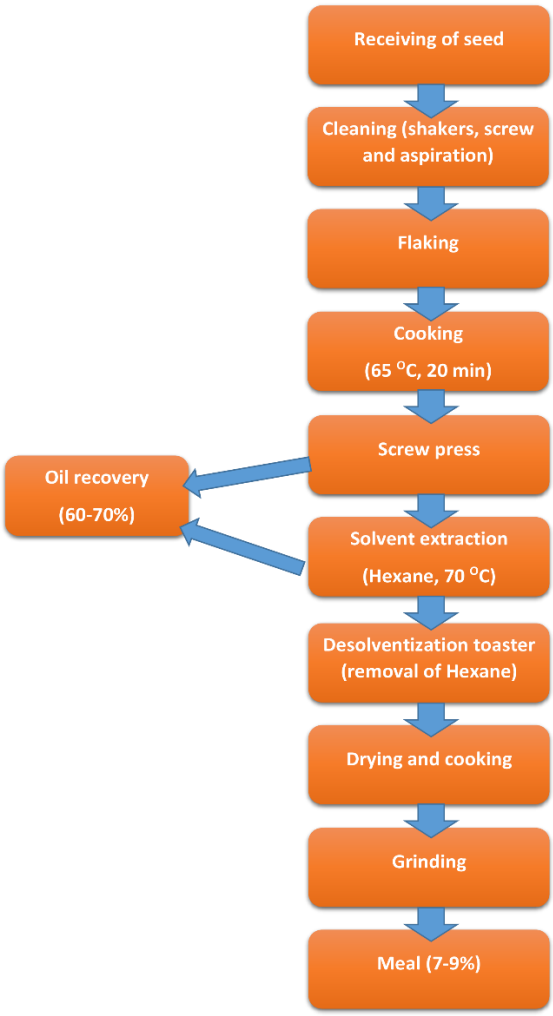


**Figure 3**: processing of flaxseed.

The amount of heat as well as extraction time influence the quality of oilseed meal as excessive heating reduce the nitrogen digestibility and net protein utilization **[50]**. During the processing of flaxseed to meal, protein, carbohydrates and mineral levels increased significantly, which contribute to decrease in lipid content. Protein solubility of seeds declined while processing to meal but substantial increase in solubility of protein were observed in flaxseed flakes which may be credited to the increased surface exposure of protein during conditioning treatment. Heat treatment affects the quality of protein and

therefore decreases the protein solubility. **[18-51]** However, the total cyanogenic contents of the meal remained unaltered by processing. Several attempts are being made from time to time to reduce the cyanogenic compounds of the flaxseeds. One of the simple and convenient methods to eliminate the cyanogenic glycosides by use of exogenous enzymes to produce hydrogen cyanide from the flaxseed meal and then subjected to steam for evaporation of hydrogen cyanide **[52]**. Similarly, autoclaving, microwave roasting, pelleting of flaxseeds resulted in significant reduction in cyanogenic glycoside content of the meal without lowering nutritional quality of the seed **[53]**.

Noting that our study is concerning flaxseed oil, therefore we will focus more on oil extraction

**II.2.2 Oil extraction:**

Commercially majority of the flaxseed is processed for extraction of oil which is then used for paints, coatings, linoleum, inks, floor coverings, etc. **[31]**. Industrial oil is not suitable for food or feed, but the residual meal can be used as feed for cattle. The very high content of alpha-linolenic acid content of flaxseed make it susceptible to autoxidation, leading to deterioration of quality, therefore flaxseed oil extraction has been done by cold pressing and solvent extraction methods. Even after cold extraction of flax oils, it is strongly recommended that oil should be stored in dark glass bottles, supplemented with antioxidants to avoid quality deterioration **[54]**. In India, various techniques are used for the extraction of flaxseed oil, namely bullock driven ghanis (Kohlu), power driven rotary ghanis hydraulic press and screw-press oil expellers **[3-4]**. Flaxseed oil is generally screw pressed without heat treatment as well no refining is done except for sedimentation and filtration **[55]**. Fresh unrefined oil has a pleasant nutty flavor and attractive golden color. The oil recovery using double stage compression screw press ranges from 86 to 92%. However, various pretreatments viz., the adjustment of moisture content **[56]**, use of enzymes **[57]** steam treatment, cooking **[3-4]** prior to pressing results in significant improvement in oil recovery. Decreasing the moisture content of the flaxseeds from 13.8 and 6.5 % resulted in significant increase in oil recovery varying from 44.4 and 81 % **[3-4]**.

Solvent extraction of oilseeds using hexane is usually carried out for recovery of high quality oil and retention of polar lipids **[58]**. Cold pressing results in only partial recovery of the oil; therefore, pressing of the seeds is followed by solvent extraction at high temperatures to achieve maximum oil recovery. However, the alpha-linolenic acid is degraded by exposure to high temperature; therefore, supercritical fluid extraction technique can be a boon to such oils. Supercritical carbon dioxide (SC-$CO_2$) is the most often used super critical fluid for

purpose of oil extraction as the low critical temperature of $CO_2$ (31°C) allows extraction of heat sensitive compounds without quality deterioration. Lipid composition of the flaxseed oils obtained by both SC-$CO_2$ and petroleum ether extraction were studied and it was found that the alpha-linolenic acid content was higher in case of the oil extracted using SC-$CO_2$ as compared to oil extracted using petroleum ether **[59]**. Ultrasonic power is also employed for the extraction of flaxseed oil. A study revealed that ultrasonic assisted extraction of oil resulted in enhanced recovery of oil with increased ultrasonic power. Ultrasonic assisted extraction saves time and lesser solvent consumption **[60]**.

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# Chapter III: Methods and materials

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## III.1 Materials and apparatus:

### III.1.1 Materials:

- Distilled water
- Neutralized Ethanol
- Diethyl ether
- Chloroform
- Hexane
- Phenolphthalein
- Sodium thiosulfate ($Na_2SO_4$)
- Gallic acid
- Potash (KOH)
- Starch powder
- Potassium iodide (KI)

### III.1.2 Apparatus:

- Heating agitator
- graduated burette + support
- Beakers
- Erlenmeyer
- Petri dishes
- Scale
- UV-spectrophotometer
- FTIR spectrophotometer
- Refract meter
- pH meter
- Pastor droppers

## III.2 Acidity:

### III.2.1 Preparations:

- **Phenolphthalein solution 1%:**

Dissolve 1g of Phenolphthalein powder in a 100 ml solution of ethanol/distilled water (1:1) in a 100 ml volumetric flask, agitate well for total dissolution (homogenous phase).

- **Potash 0.1N solution:**

In a 1 liter beaker, dissolve 5.6g of dry potash in 1000ml of distilled water, agitate well for total dissolution (homogenous phase).

### III.2.2 operating protocol:

In a 200 ml Erlenmeyer, weigh 1g of linseed oil to be analyzed then add 50 ml of ethanol/diethyl ether mixture (1:1), then with a pastor dropper add three to four drops of Phenolphthalein, after that heat the mixture using a heating agitator for 10 minutes until total dissolution with a proper heating (35°C).

At last, the solution is to be titrated with a 0.1N potash solution with continuous agitation until the appearance of a persistent color (15 seconds). Note the titration volume.

The acidity of a greasy corps is to be determined with the following law:

$$A\% = \frac{282.5 * N * V * 100}{m * 10}$$

- A%: acidity (percentage)
- V: the titration volume of KOH (ml).
- m: sample weight (mg).
- N: the normality of KOH solution (0.1N).

**Figure 1:** titration montage


**Figure 2:** acidity color variation

## III.3 Peroxide value:

### III.3.1 Preparations:

- **Starch solution 1%:**

In a 100 ml volumetric flask, dissolve 1g of starch powder in distilled water.

Place the flask on a heating agitator and mix with heat until total dissolution (homogenous phase).

- **Sodium thiosulfates solution (0.01N):**

In a 1 liter beaker, dissolve 2.4g of sodium thiosulfates powder in 1 liter of distilled water, agitate well until total dissolution (homogenous phase).

- **Potassium iodide solution:**

In a 100 ml volumetric flask, dissolve 2g of KI powder in 50 ml of distilled water, agitate until total dissolution (homogenous phase).

**III.3.2 operating protocol:**

Weigh 2g of linseed oil to the nearest 0.01g (as accurate as possible) in a 200 ml Erlenmeyer, then add 10 ml of chloroform followed with 15 ml of glacial acetic acid.

After that introduce 1 ml of the prepared potassium iodide solution into the mixture, manually shake the solution then hide it from light for 5 minutes.

Then add 75 ml of distilled water to the mixture along with 3 to 4 drops of the 1% starch solution via pastor dropper.

Finally you get to titrate the previous mixture with the 0.01N solution of sodium thiosulfates slowly in high agitation until you get a change of color.

Mark the volume used in titration.

Repeat the procedure without any oil sample.

The peroxide value is to be determined with following law:

$$POV = \frac{(V - V_0) * N}{m} * 1000$$

- POV: peroxide value $meq(g)_{O2}/Kg_{(oil)}$.
- V: titration volume of $Na_2SO_4$ solution for oil test.
- $V_0$: titration volume of $Na_2SO_4$ solution for blank test.
- N: normality of $Na_2SO_4$ titration solution (0.01N).
- m: sample weight (g).

**Figure 3:** peroxide value titration result

**Figure 4:** Blank test result

## III.4 Water rate:

First of all, heat a petri dish in the oven for one day on high temperature (120 °C) to make sure that all water is no more, after that weigh the dish in use on a scale and mark its weight ($m_0$), add a sufficient quantity of linseed oil into the petri dish then weigh it again ($m_1$), introduce the oil dish into the oven for 15 minutes under 110~115 °C.

At last weigh the oil dish after heating and mark it weight ($m_2$).

Water rate of a greasy corps/oil is to be determined with the following law:

$$H\% = \frac{m_2 - m_1}{m_2 - m_0}$$

- H%: water rate
- $m_0$: petri dish weight
- $m_1$: petri dish + oil before heating
- $m_2$: petri dish + oil after heating

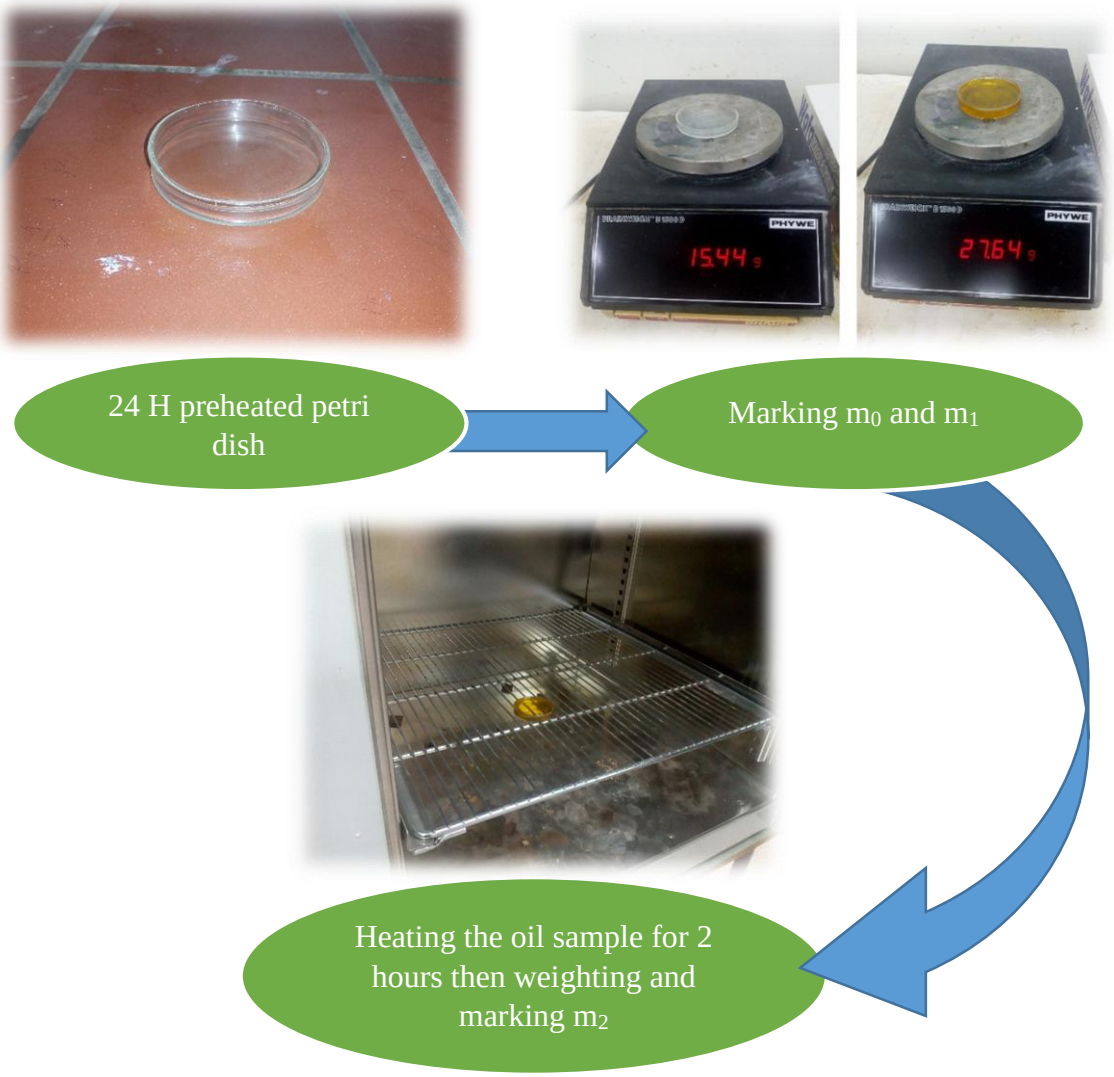


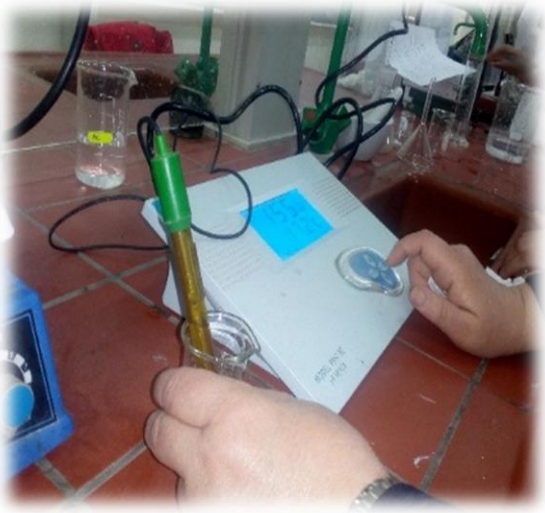

**Figure 5:** water rate measuring protocol

## III.5 pH:

First it's necessary to calibrate the pH meter in order to get a proper read, we get to use standard buffer solutions depending on the solution pH interval, and for linseed oil we use neutral then acid buffer solution by order due to the nature of linseed oil:


Buffer solutions 4.01, 70.1, 11.1 by order

Calibration via buffer solution ($T^O$ is considered)



Waiting for pH value to stabilize after cleaning and plunging the sensor into the oil sample

**Figure 6:** linseed oil pH measuring

## III.6 refractive index:

First you need to clean the refract meter using absorbent paper. Next you calibrate the apparatus with distilled water ($n_{water}^{20^0C} = 1.33$). After that clean the refract meter slide once again via absorbent paper.

Finally you get to place a few drops of oil to be analyzed in the refract meter slide and adjust the slide circle of dark and clear chamber in half, make the reading results taking into account the temperature.

$$n_d^{20^0C} = n_d^T + 0.00035(T - 20)$$

**Figure 7:** Refract meter PI model

- $n_d^{20^0C}$: Refraction value ($T^0$= $20^0$C).
- $n_d^T$: Refraction value ($T^0$ during analysis).
- T: Temperature of the sample during analysis.
- 0.00035: Refraction value variation of triglycerides with degree next to $20^0$C.

## III.7 Spectral analysis:

### III.7.1 FTIR infrared spectral analysis:

For this analysis we have a FTIR-8400 S Shimadzu model in hand:


**Figure 8:** FTIR-8400 S Shimadzu infrared spectrophotometer

Performing analysis is as the following:

Get your main oil sample alongside with a fluid sample support for IR analysis with pastor dropper:

Open the support:

Add a few drops of oil with pastor dropper into the support:

Close the support and introduce it to the spectrophotometer:

Finally you get to perform your FTIR analysis by launching the specified software (Shimadzu IR Solution) via PC and you get your spectrum.

**III.7.2 Ultraviolet spectral analysis:**

For this analysis we have an UviLine 9400 SECOMAM UV spectrophotometer model in hand:

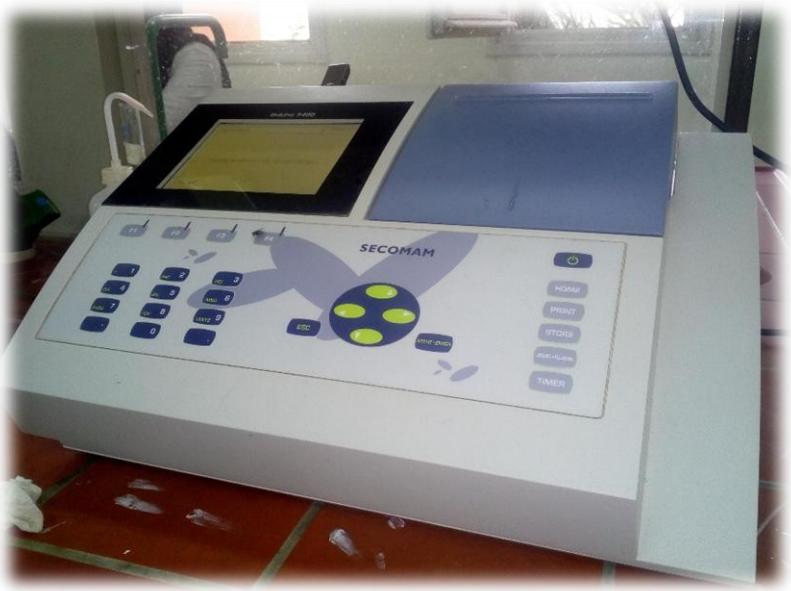
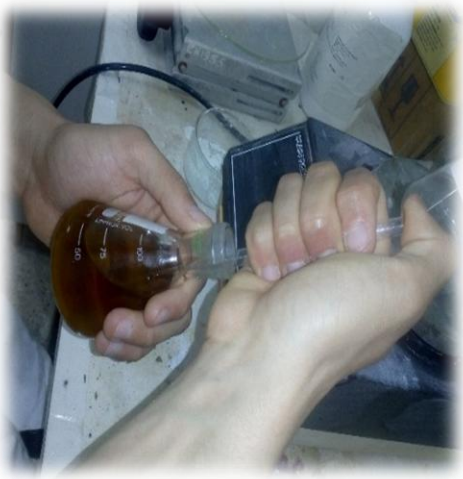



**Figure 9:** UviLine 9400 SECOMAM UV spectrophotometer

First of all we launch the spectrophotometer and let it heat until it is ready (commonly for 15 to 20 minutes).

Now we prepare our sample:

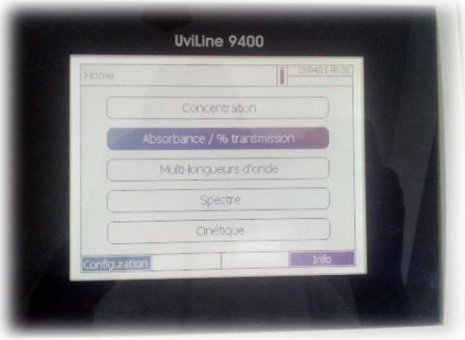
Weigh the desired mass of oil to be analyzed (0.1g) in a 200 ml Erlenmeyer over a balance scale:



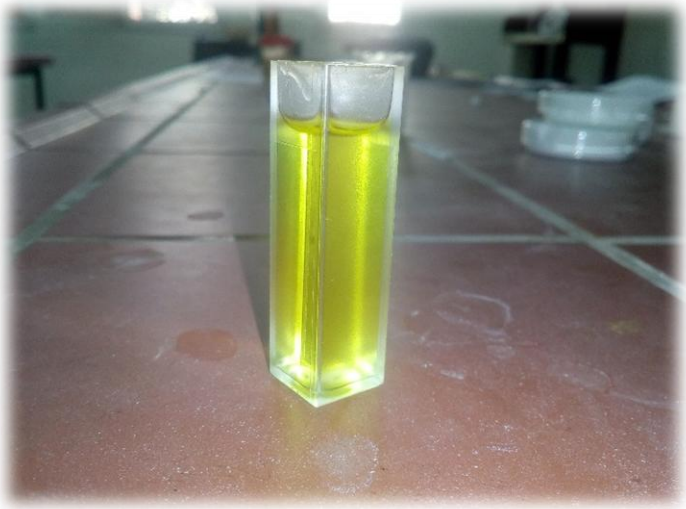
After that, we introduce 10 ml of hexane into the Erlenmeyer:

Agitate manually until total dissolution (homogeneous phase):

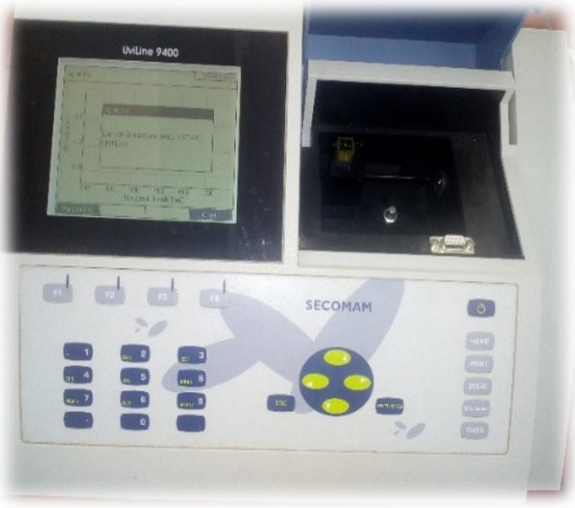
Select absorbance/transmittance from the menu, set your wavelength in the spectrophotometer, and fill a glass cuvette (Quartz cuvette for better result) with hexane to set your blank:




Fill another glass cuvette with the prepared solution:



Introduce the cuvette into the spectrophotometer and close the hood:



Hit the start button and wait until the results show up:


## III.8 flaxseed oil sampling for continuous evaluation:

For this part, we bring five equal volumes of flaxseed oil (100ml) and mark them as the following:

L1: this sample goes to open air in the presence of sunlight without any additives.

L2: this sample is to be prepared by dissolving 1g of Gallic acid in the 100ml of flaxseed oil; this sample goes to open air in the presence of sunlight.

$G_1$: this sample is to be prepared by dissolving 0.1g of Gallic acid in the 100ml of flaxseed oil; this sample is isolated from both air and any light.

$G_2$: this sample is to be prepared by dissolving 0.3g of Gallic acid in the 100ml of flaxseed oil; this sample is isolated from both air and any light.

$G_3$: this sample is to be prepared by dissolving 0.7g of Gallic acid in the 100ml of flaxseed oil; this sample is isolated from both air and any light.


**Figure 10:** L1-L2-G1-G2-G3 linseed oil samples by order

# Chapter IV: Discussing results

# Chapter IV: Discussing results

## IV.1 Physiochemical and organoleptic parameters:

A good oil has a low acidity level, which gives it greater stability against oxidation by air. This is even more important if the oil has a high-unsaturated fatty acid content. The factors responsible for high acidity are related to non-compliance with good harvesting and manufacturing practices for flax oil.

Noting that for this thesis, we used “El Captain Company (CAP PHARM)” commercial flaxseed oil:


The peroxide value is used to evaluate the oxidation state of the oil. Chemical alteration of oils is caused by oxidation in the air resulting in the formation of peroxides.

This index could be used to control the quality of the oils, as it depends on the problems that may occur after harvesting (storage conditions of the seeds before treatment and during processing).

The phenomenon of oxidation of fatty acids leads to changes in the organoleptic, chemical and nutritional properties. These alterations affect the marketability of the product.

It’s a parameter that determines the degree of unsaturation of fatty acids in the composition of fats. it varies with the wavelength of incident light and temperature.

The pH intervenes on the mechanism of oxidation of lipids. Oils with a pH below four are acidic oils. Storage in the open air modifies the pH. This parameter allows the total elimination of water and volatile products.

**Table 1:** physicochemical Parameters

| Value | Measured | Norms | Method |
|---|---|---|---|
| **Acidity (%)** | 0.36725 | Max 2.00 | NF EN ISO 660 |
| **Peroxide value (meq $O_2$/Kg oil)** | 2 | Max 15.00 | NF EN ISO 3960 |
| **pH** | 3.7 | / | / |
| **Water rate (%)** | 0.0037 | Max 0.1 | Ph. Eur 2.5.32 |
| **UV Absorbance 232 nm** | 0.224 | / | / |
| **UV Absorbance 270 nm** | 0.733 | / | / |
| **Refractive index** | 1.48575 (T=25°C) | 1.478 – 1.483 (ND T=40°C) | ISO 6320:2000 AOCS Cc 7-25 AOAC 921.08, IUPAC 2.102 |

**Table 2:** Organoleptic parameters.

| Aspect | Color | Scent | Taste | Flavor |
|---|---|---|---|---|
| Liquid, clear | Gilded yellow | Medium | Good | Sour |

The results show that the studied linseed oil has a water content below the standards. The humidity, leads to an increase in the growth of yeasts and molds during storage.

Regarding the absorbance in the ultraviolet (A), it can provide indications on the quality of a fat, on its state of modification due to technological processes.

The results show that our oil has ultraviolet absorbance values below the standards. Therefore, the oil does not contain secondary products such as linoleic hydroxyperoxide, unsaturated ketones and diketones.

Results are related to several factors such as late harvesting of the seeds, excessive exposure of the seeds and the extracted oil to oxygen in the air and to light.

The results obtained in **Tables 1** and **2** allow us to say that:

- The flaxseed oil studied is not very acidic and is suitable for consumption.
- The peroxide value is in agreement with the one quoted in the standards, which allows qualifying it as good.

- The refractive index is in accordance with the standards; it is therefore totally pure.
- The water content is within the standards.
- The absorbance in the ultraviolet meet the standards.
- The organoleptic characteristics show that the studied linseed oil does not present any anomaly.

## IV.2 Chemical analysis:

### IV.2.1 Acidity:

The acidity values of the 5 samples at different concentrations and as a function of storage time, are given in **Table 3** and illustrated in **Figure 1**

**Table 3:** Evolution of acidity

| | 0 days | 10 days | 20 days | 30 days | 40 days | 50 days | 60 days |
|---|---|---|---|---|---|---|---|
| L1 | 0.36725 | 0.452 | 0.59325 | 1.83625 | 2.8645 | 2.933 | 3.211 |
| L2 | 0.36725 | 1.017 | 1.04525 | 2.147 | 2.79675 | 2.688 | 2.125 |
| G1 | 0.36725 | 0.5085 | 0.678 | 1.66675 | 2.712 | 2.725 | 2.951 |
| G2 | 0.36725 | 0.67872 | 0.904 | 1.72325 | 2.895625 | 2.866 | 2.619 |
| G3 | 0.36725 | 0.8475 | 0.98875 | 1.793875 | 2.938 | 3.033 | 2.929 |

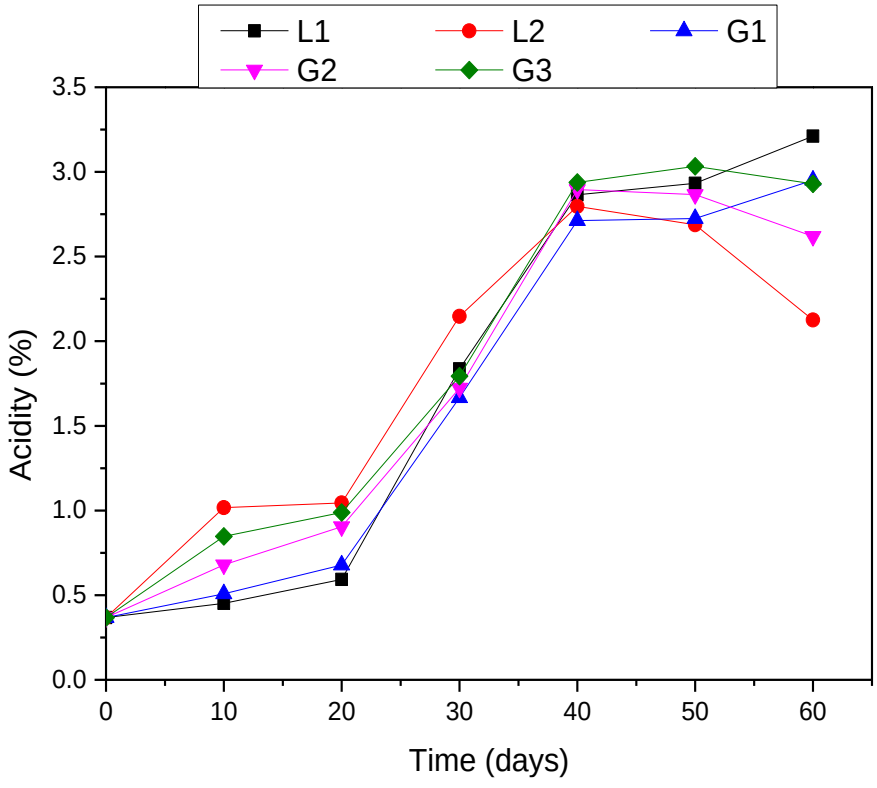



**Figure 1:** Acidity plot

It is noted that the initial acidity of our oil, which is 0.367%, is consistent with the limits established by the literature.

From the results recorded in **Table 3** and reported in **Figure 1**, we notice that all the acidity values of the flaxseed oil samples to which Gallic acid is added seem to have the highest values due to the acidic function of the antioxidant used for these samples. This variation in acidity (increase) is slight for all sets of oil samples used.

In all cases, the acidity of the oil studied varied significantly with the addition of antioxidants. These results are not in agreement with those obtained by N.Denisse **[1]** who found that the acidity of sunflower, walnut and soybean oils to which 80 ppm and 160 ppm of phenolic extract of margins are added remains constant during a storage period of 22 days at 60°C. This trend was also noticed by S.Fodil **[2]** when studying the effect of β carotene and vitamin E on the oxidative stability of three types of virgin olive oils.

**IV.2.2 Peroxide value:**

**Table 4** and **Figure 2** give the distribution of peroxide value of linseed oil samples according to the mass of added materials as a function of storage time.

**Table 4:** Evolution of peroxide value

| | 0 days | 10 days | 20 days | 30 days | 40 days | 50 days | 60 days |
|---|---|---|---|---|---|---|---|
| **L1** | 2 | 13 | 16.5 | 18 | 21 | 21.5 | 32 |
| **L2** | 2 | 7 | 5 | 4.7 | 5 | 6.5 | 6.5 |
| **G1** | 2 | 1 | 2.5 | 4 | 5 | 6.25 | 6 |
| **G2** | 2 | 2.5 | 2 | 4 | 3.7 | 3.5 | 3.5 |
| **G3** | 2 | 3.5 | 1.25 | 1.25 | 2.5 | 4 | 5.5 |

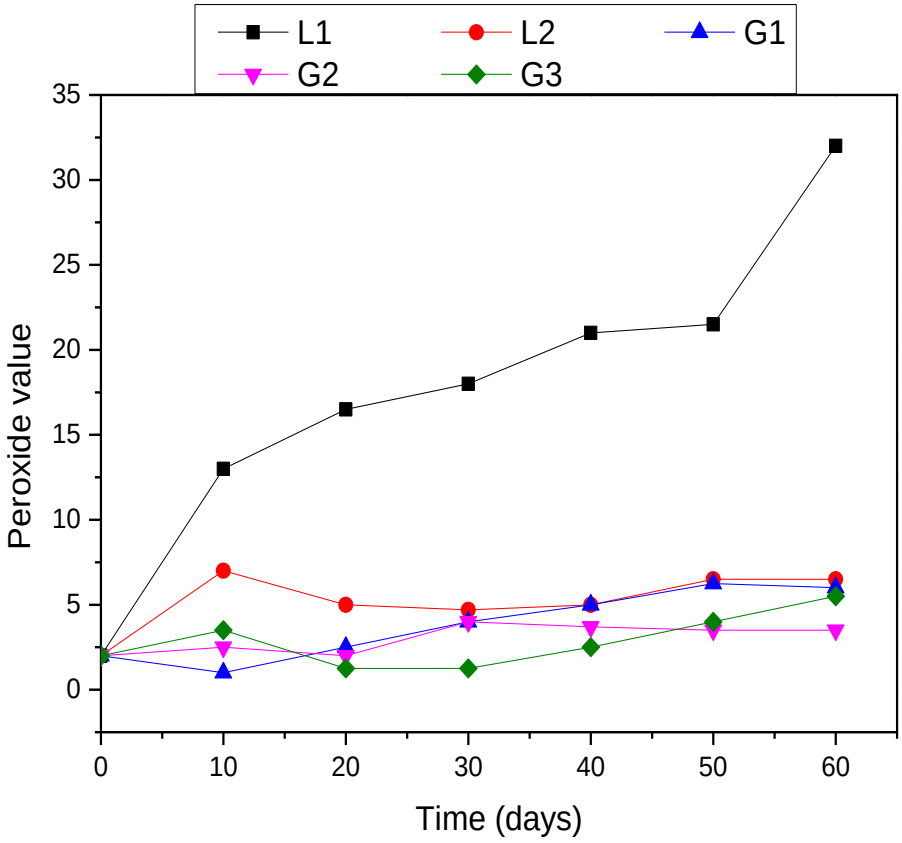



**Figure 2:** Peroxide value plot.

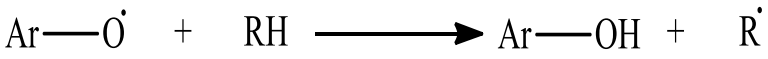
The first products formed by the attack of oxygen, activated on the double bonds of fatty acid chains, are unstable peroxidized compounds, and hydroperoxides whose structure will depend on the nature of fatty acids attacked (mono-, di-, tri- or polyunsaturated acids).

The determination of the peroxide value is the most appropriate method for the measurement of these peroxidized compounds.

The analysis of the results of **Table 4** represented in **Figure 2** shows an evolution of the peroxide index of all the series of samples as well as that of the two controls according to the duration of storage.

The initial value of the peroxide value of the control oil sample exposed to air and sunlight is 2meq/kg. After 40 days of storage, it reaches a value of 21 meq/kg and 32meq/kg after 60 days. The other oil samples to which the antioxidants used in this work are added, as well as the control sample exposed to the light, seem to be better protected against oxidation than the control exposed to the sun. The peroxide values obtained after 40 days of storage ranged from 3 meq/kg to 6 meq/kg respectively for the samples with added Gallic acid. They correspond to optimal values for the protection of linseed oil from oxidation. While the results found with the incorporation of Gallic acid are optimum with the sample (G2), this

is in agreement with the results of F. Pirisi et al **[3]** who showed that the oxidation stability, evaluated by the Swift test with Rancimat, would not be correlated with the phenolic compounds content. Similarly, for Cillard et al **[4]** who found that increasing concentrations of antioxidant added in the medium could be responsible for a prooxidant effect, as it has been demonstrated for α-tocopherol. In fact, if the concentration of the radical form of the antioxidant produced by oxidation increases significantly, it can act as an initiator of lipid peroxidation according to the reaction proposed by H. Chimi et al **[5]**:


From the twentieth day of storage, the peroxide value of the samples increases with time.

We note that the flaxseed oil samples with the synthetic antioxidant (Gallic acid) incorporated are the least peroxidized, with a value of the peroxide index at the end of the shelf life (60 days) equal to 3.5meq/kg for the mass (G2 = 0.3g) of Gallic acid.

These results can be explained by the synergistic effect and by the structure of Gallic acid, which has three phenol functions that can also give up three hydrogen atoms to peroxyl radicals (RO•) and hydroperoxyl radicals (ROO•). Consequently, it is likely to stabilize three radical functions. In conclusion, we can say that the peroxide value represents one of the quality parameters of linseed oil, but cannot be an indicator of the oxidative stability of the oil.

## IV.3 monitoring the oxidation of linseed oil by physical method:

### IV.3.1 Absorbance to UV radiation:

All fats contain epoxides and hydroperoxides in varying amounts. Isomerization reactions lead to the formation of conjugated dienes and trienes which absorb light between 225 and 280nm **[6]**. Indeed, conjugated dienes and primary products of fatty acid oxidation formed by rearrangement of the double bonds of the alkyl radical of polyunsaturated fatty acids when they have a conjugated diene structure absorb light near 232 nm. Conjugated trienes (in the case of fatty acids with three conjugated double bonds) and oxidation by-products, such as α-unsaturated aldehydes and ketones, absorb light at about 270 nm. The determination of absorbances in the vicinity of these two values allows the detection and evaluation of the quantities of oxidation products: the higher the extinction at 232 nm, the

more peroxidized it is. Similarly, the higher the absorbance at 270 nm, the higher the amount of secondary oxidation products **[7]**.

**Table 5** and **Figure 3** give the distribution of absorbance values at 232 nm of linseed oil samples as a function of storage time.

**Table 5:** Evolution of UV absorption (λ= 232nm)

| | 0 days | 20 days | 30 days | 40 days | 50 days | 60 days |
|---|---|---|---|---|---|---|
| **L1** | 0.224 | 0.289 | 0.224 | 0.219 | 0.480 | 0.497 |
| **L2** | 0.224 | 0.252 | 0.364 | 0.214 | 0.468 | 0.444 |
| **G1** | 0.224 | 0.251 | 0.249 | 0.366 | 0.380 | 0.390 |
| **G2** | 0.224 | 0.238 | 0.252 | 0.305 | 0.342 | 0.339 |
| **G3** | 0.224 | 0.303 | 0.262 | 0.401 | 0.389 | 0.410 |

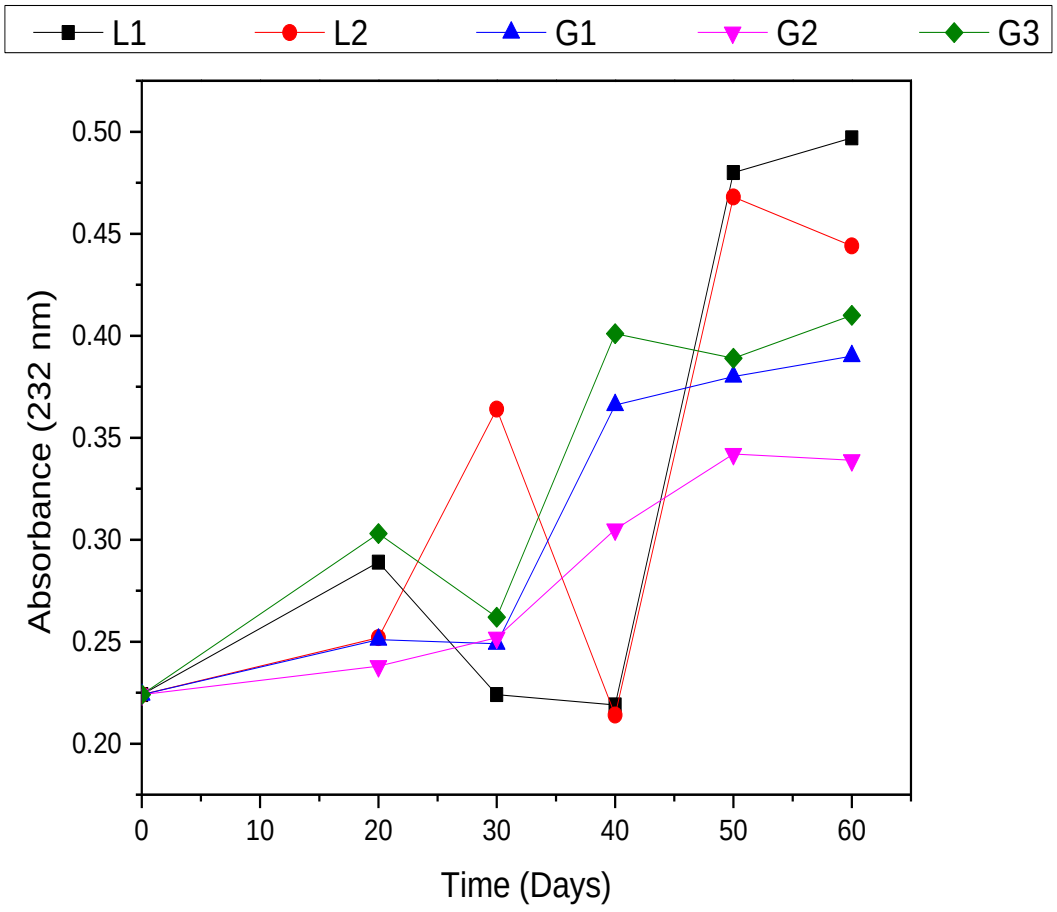



**Figure 3:** UV absorption plot (λ= 232nm)

According to **Table 5** and **Figure 3**, the smallest values are observed in the series of linseed oil containing Gallic acid. On the other hand, all the samples of series A and series B as well as the control show a slight increase during the whole storage period. They are thus at the first stage of propagation which corresponds to the formation of peroxides but not of hydroperoxides.

Lipid oxidation increases with the concentration of compounds resulting from hydroperoxide degradation, this is confirmed by an increase in absorbance values at 270 nm. The extinction at 270 nm allows us to determine the proliferation of the oxidation, the secondary products of oxidation and in particular the α-diketones. This evolution of absorbance for the flax oil samples is given by **Table 6** and presented by **Figure 4**

**Table 6:** Evolution of UV absorption (λ= 270nm)

| | 0 days | 20 days | 30 days | 40 days | 50 days | 60 days |
|---|---|---|---|---|---|---|
| **L1** | 0.733 | 0.758 | 0.760 | 0.795 | 0.811 | 0.956 |
| **L2** | 0.733 | 0.732 | 0.747 | 0.721 | 0.778 | 0.859 |
| **G1** | 0.733 | 0.733 | 0.741 | 0.675 | 0.762 | 0.767 |
| **G2** | 0.733 | 0.679 | 0.720 | 0.671 | 0.642 | 0.652 |
| **G3** | 0.733 | 0.724 | 0.730 | 0.746 | 0.772 | 0.775 |

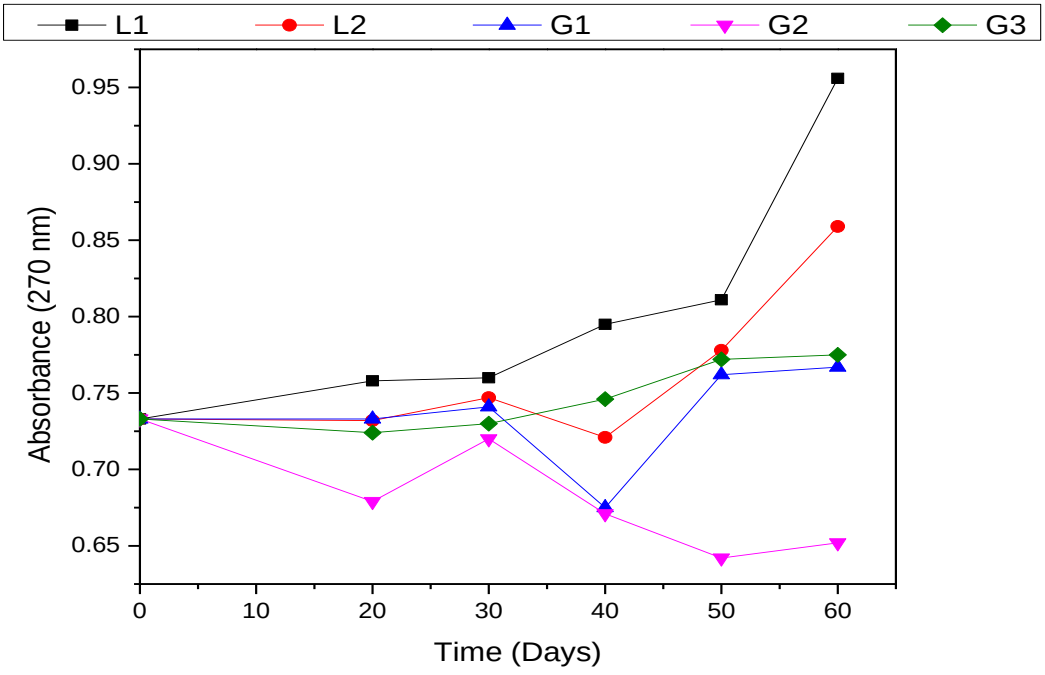


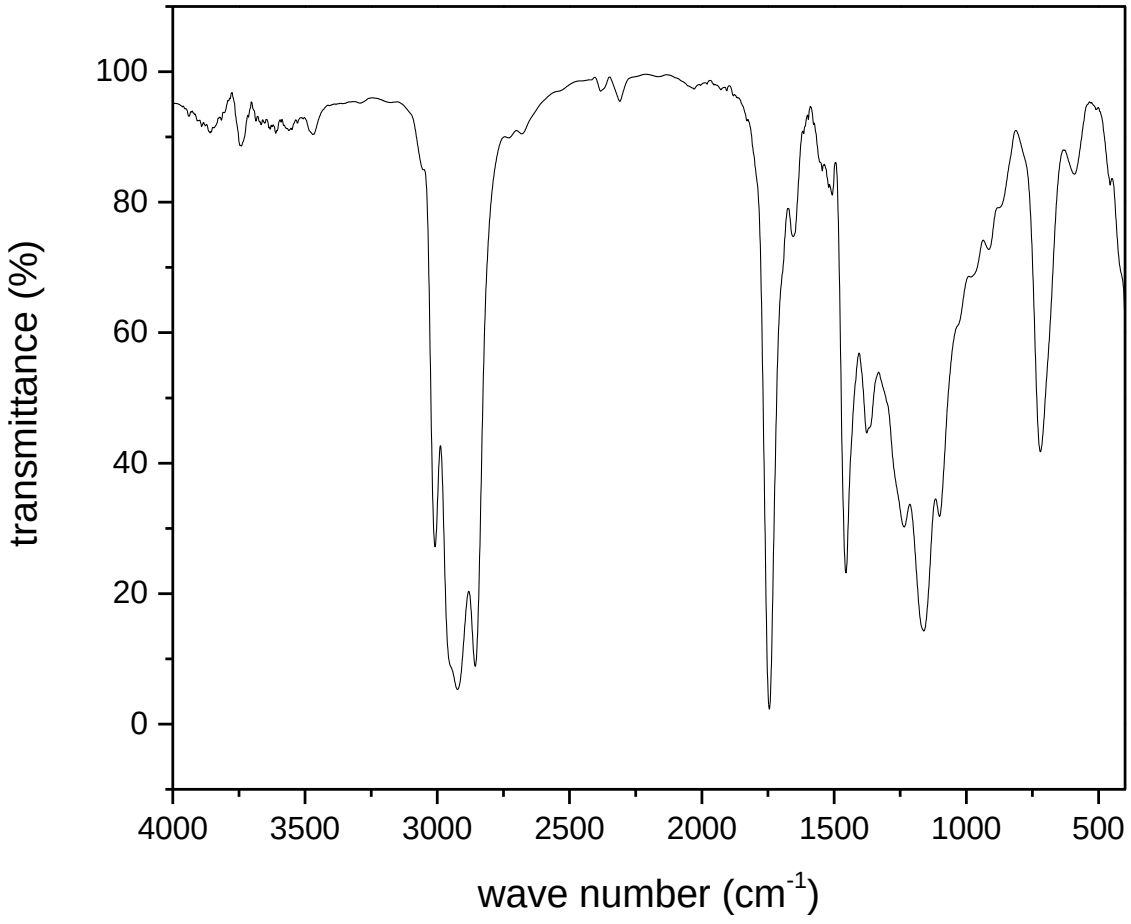

**Figure 4:** UV absorption plot (λ= 270nm)

## IV.4 Monitoring the degradation of linseed oil exposed to air and sun:

### IV.4.1 Characterization of linseed oil by FTIR spectroscopy:

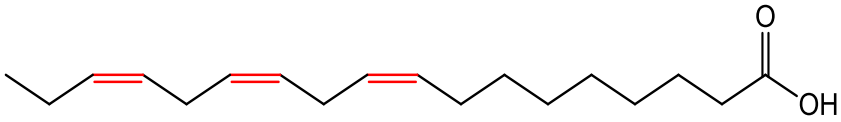
The bands observable in the region 2930 to 2800 $cm^{-1}$ are attributable to the C-H bond elongation vibrations of the $CH_2$ and $CH_3$ groups. Two intense bands at 2936 and 2853 $cm^{-1}$ are attributable to asymmetric and symmetric elongation vibrations of the $CH_2$ group, respectively. In the low frequency region, only the deformation vibration of the $CH_2$ group is observable. The bands at 1448 and 1379 $cm^{-1}$ are attributable to out-of-plane shear strain vibrations. The clearly observable band in this region is at 1165 $cm^{-1}$, this band is attributable to the elongation vibration of the C-O group of esters.

The FTIR spectrum of linseed oil shows a peak of weak intensity at 3010 $cm^{-1}$ corresponding to cis (Z) configuration =CH, and another band situated in the interval of 713 $cm^{-1}$ which also attributes the cis(Z) configuration =CH. On the other hand, the spectrum does not show a characteristic CH peak of double bonds in trans (E) configuration at 970 $cm^{-1}$. Therefore, all double bonds in virgin linseed oil are in cis configuration. Also, the spectrum clearly shows the very intense band at 1742 $cm^{-1}$ indicating the existence of the C = O bond of the esters.

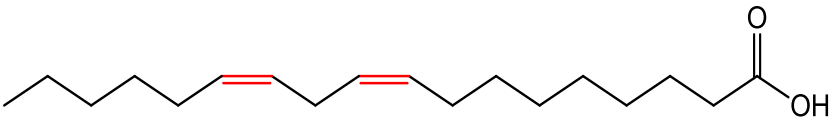


Therefore, the triglycerides of flaxseed oil are composed of three unsaturated fatty acids whose dominant isomer is "cis":

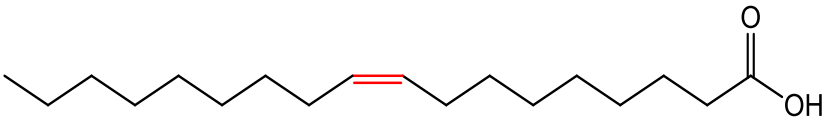
**Linolenic acid (Ln): $C_{18:3}$**




**Linoleic acid (L): $C_{18:2}$**




**Oleic acid (O): $C_{18:1}$**




These results are in perfect agreement with the results of Ornella Zovi **[8]**

The FTIR spectrum of the oil exposed to air and sun for 60 days (**Figure 5**) has the profile of a spectrum where all functional groups decline and then intensify after 20 days.

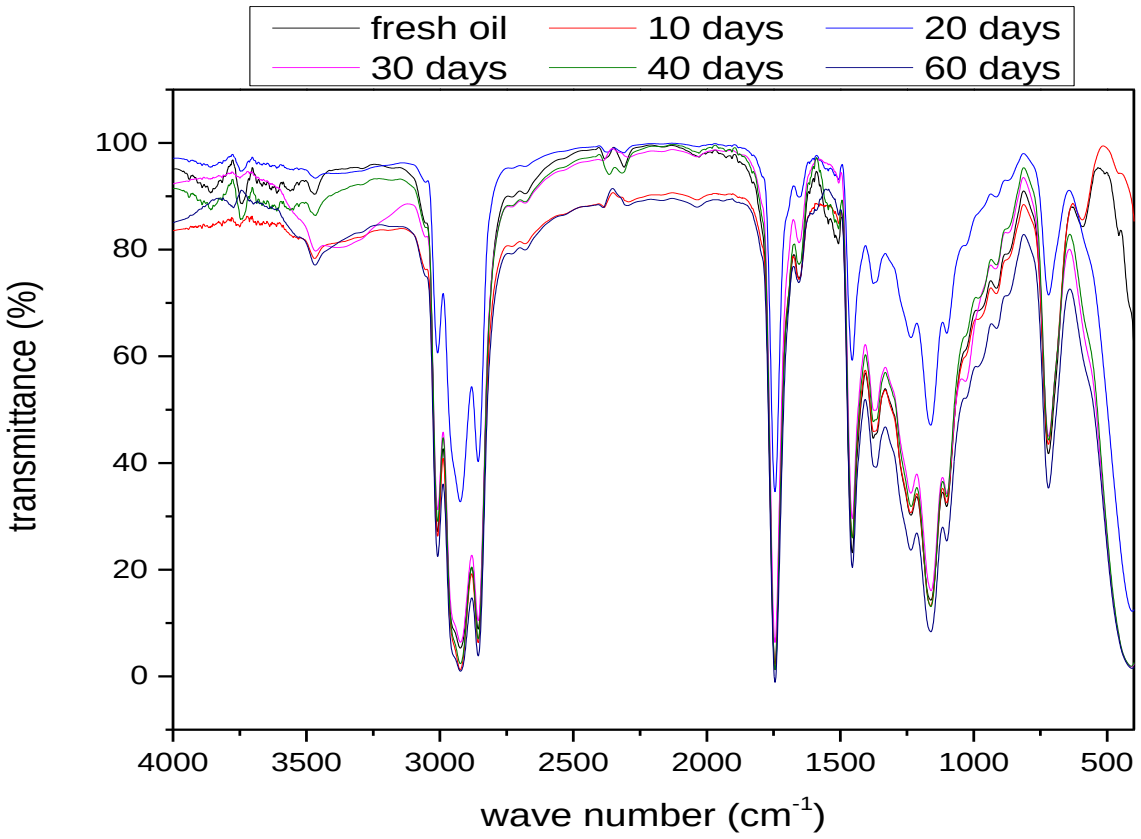



**Figure 5:** FTIR spectrum of linseed oil exposed to air and sun

We note an evolution of intensity of the absorption bands in the regions: 2954 $cm^{-1}$, 2924 $cm^{-1}$ and 2852 $cm^{-1}$. They correspond respectively to the asymmetric elongation vibration of the $CH_3$ group, the asymmetric elongation vibration of the $CH_2$ group and the symmetric elongation vibration of the $CH_2$ group.

At 1750 $cm^{-1}$, we observe that the elongation vibration of the carbonyl groups increases after the first 20 days. This effect would be related to the degree of oxidation of the oil and the relative length of the carbon chains.

In the low frequency region, a band related to the vibration of the C-O ester group is observed around 1150 $cm^{-1}$, the absorbance intensity of this band also increases after the first 20 days, which means that the intensity of this band is related to the degree of oxidation and the relative length of the carbon chains. The same can be said about the band at 724 $cm^{-1}$, which shows an intensity evolution that can easily be related to the chain length and the degree of oxidation. This band is representative of the deformations of the $CH_2$ group. As the time of exposure to air and sun increases, after the first 20 days, the carbon chain of the fatty acids in the oils regains its length and therefore the absorbance intensity of this band increases again.

**IV.4.1.1 the "cis" elongation and "trans" deformation vibration bands as well as the deformation vibration band of the =C-H group:**

The degree of unsaturation of the oil is represented by a peak around 3010 $cm^{-1}$ in **Figure 6** and at 720 $cm^{-1}$ as shown in **Figure 7**, attributable to the elongation vibration of the =C-H "cis" group and by a peak around 972 $cm^{-1}$, attributable to the deformation vibration of the =C-H "trans" group. The figure shows that the peak corresponding to the =C-H "cis" group intensifies, always, during exposure to air and sun until the 60th day and becomes almost stable.

No appearance of the "trans" isomer around 972 $cm^{-1}$, which means that the amount of the "cis" isomer remains unchanged.as demonstrated in **Figure 8.**

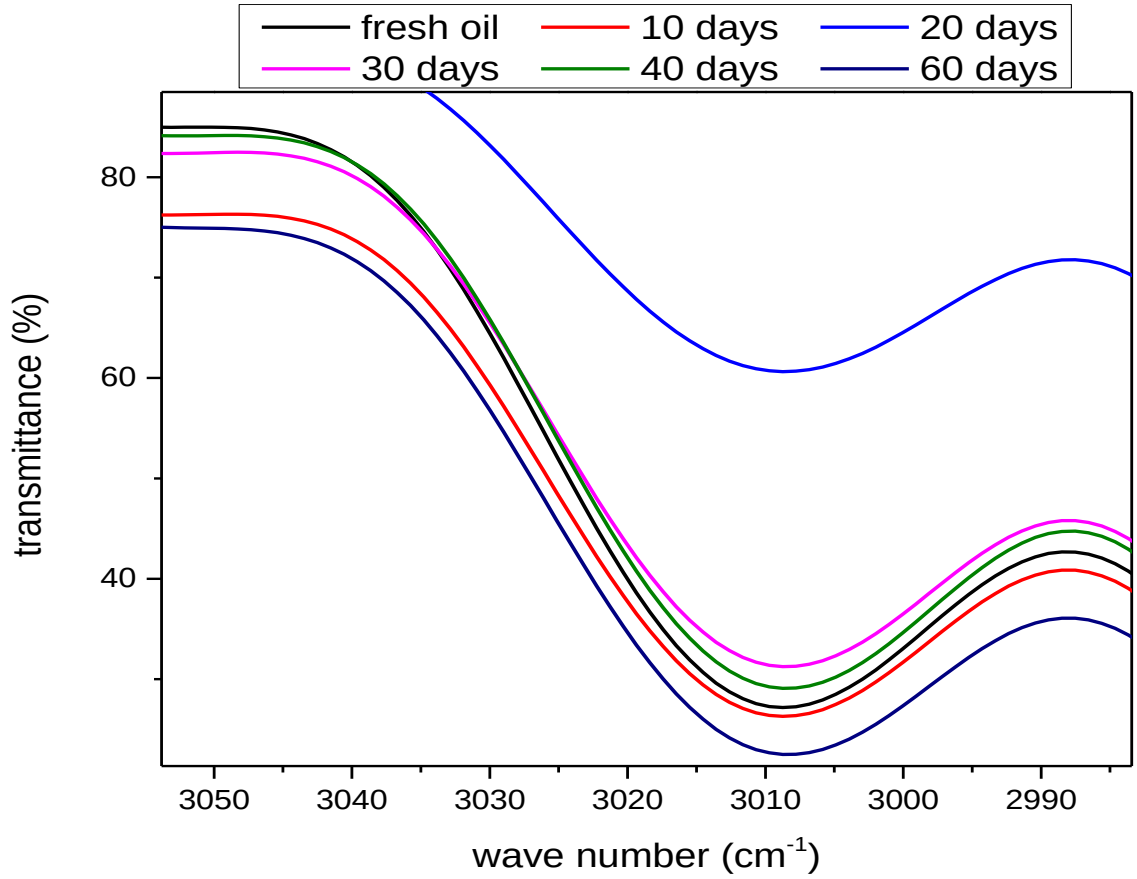



**Figure 6:** Cis C=H evolution at 3010 $cm^{-1}$ (Zoom of Figure 5)

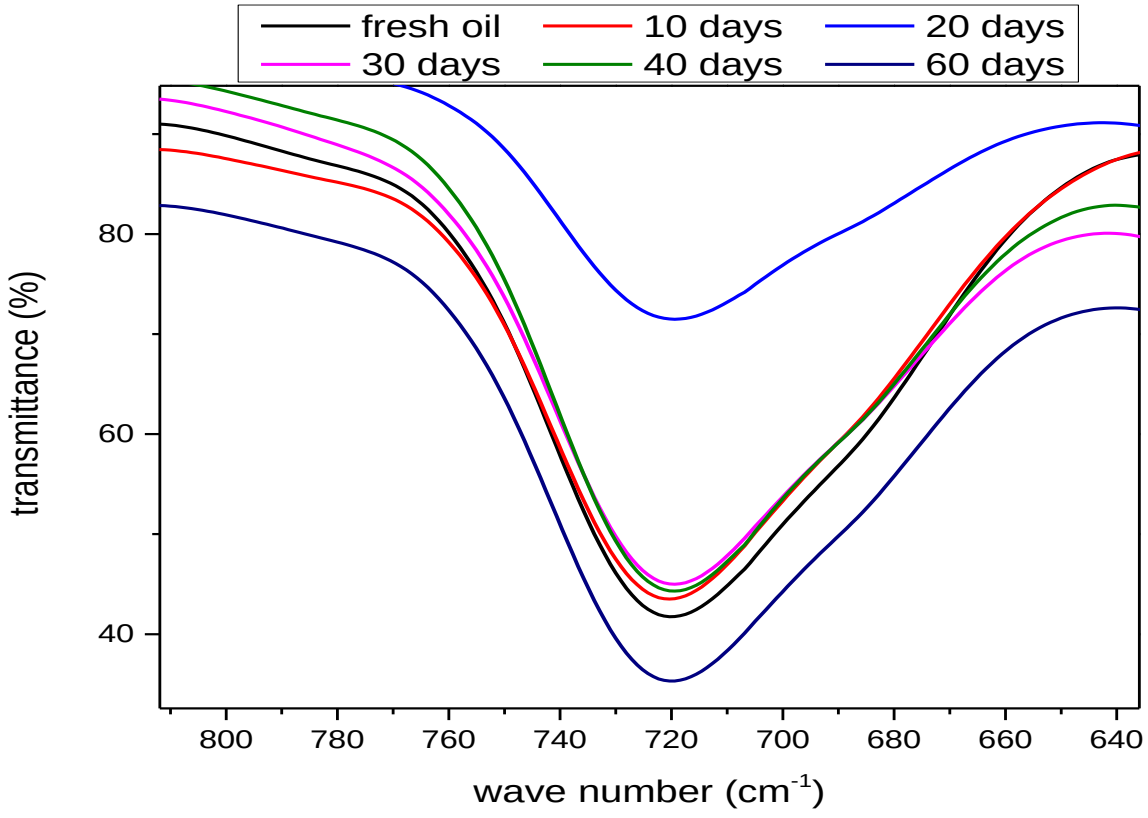



**Figure 7:** Cis C=H evolution at 720 $cm^{-1}$ (Zoom of Figure 5)

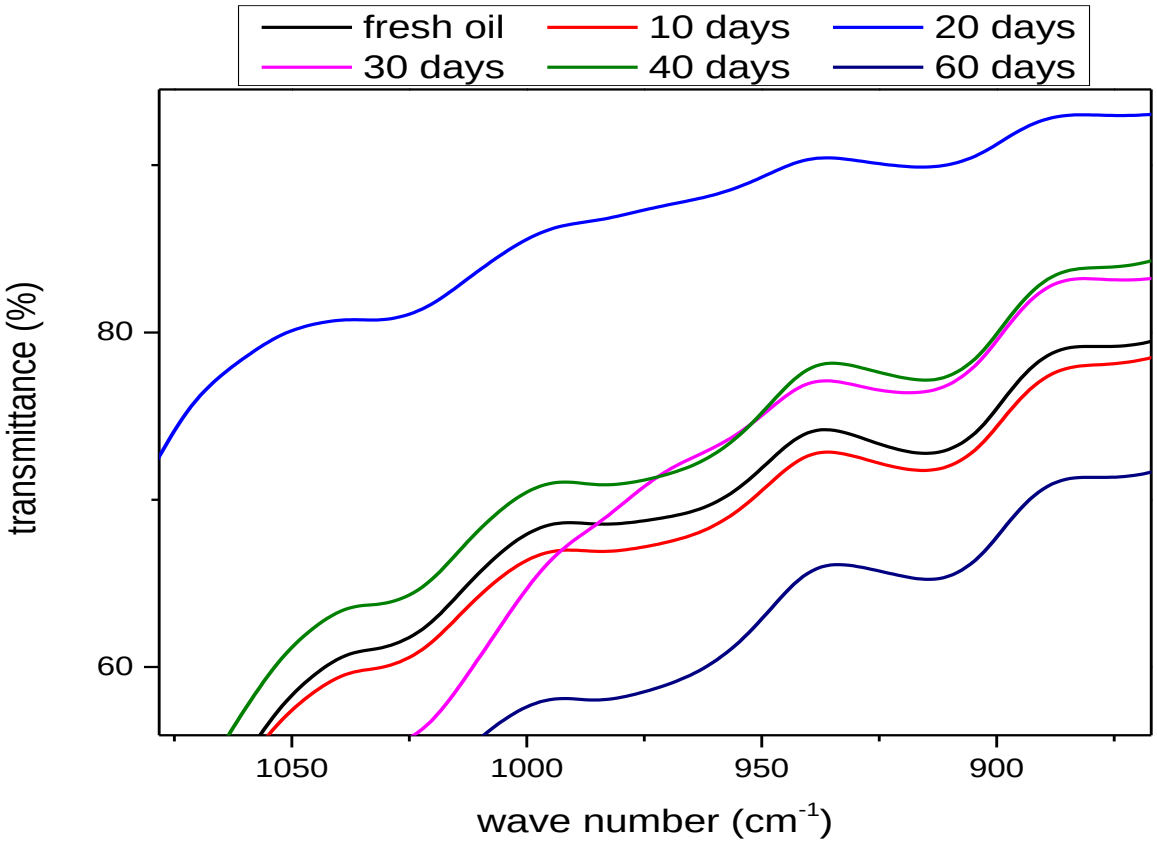



**Figure 8:** No appearance of Trans C=H isomer between 980-970 $cm^{-1}$ (Zoom of Figure 5)

**IV.4.1.2 the vibrational bands of asymmetric and symmetric elongations of the CH2 group and symmetric of the CH3 group:**

**Figure 9** shows that the absorbance intensity of the two peaks at 2924 $cm^{-1}$, at 2854 $cm^{-1}$ and the peak around 2958 $cm^{-1}$ attributed respectively to the asymmetric and symmetric elongation vibrations of the $CH_2$ group of the oils and to the asymmetric vibrations of the $CH_3$ group. Decreases during the first 20 days and then the increase in their intensity will take place and stabilize. This could be the result of a progressive decrease in the carbon chain length of the fatty acids of the oil and then the absorbance resumes its intensity.

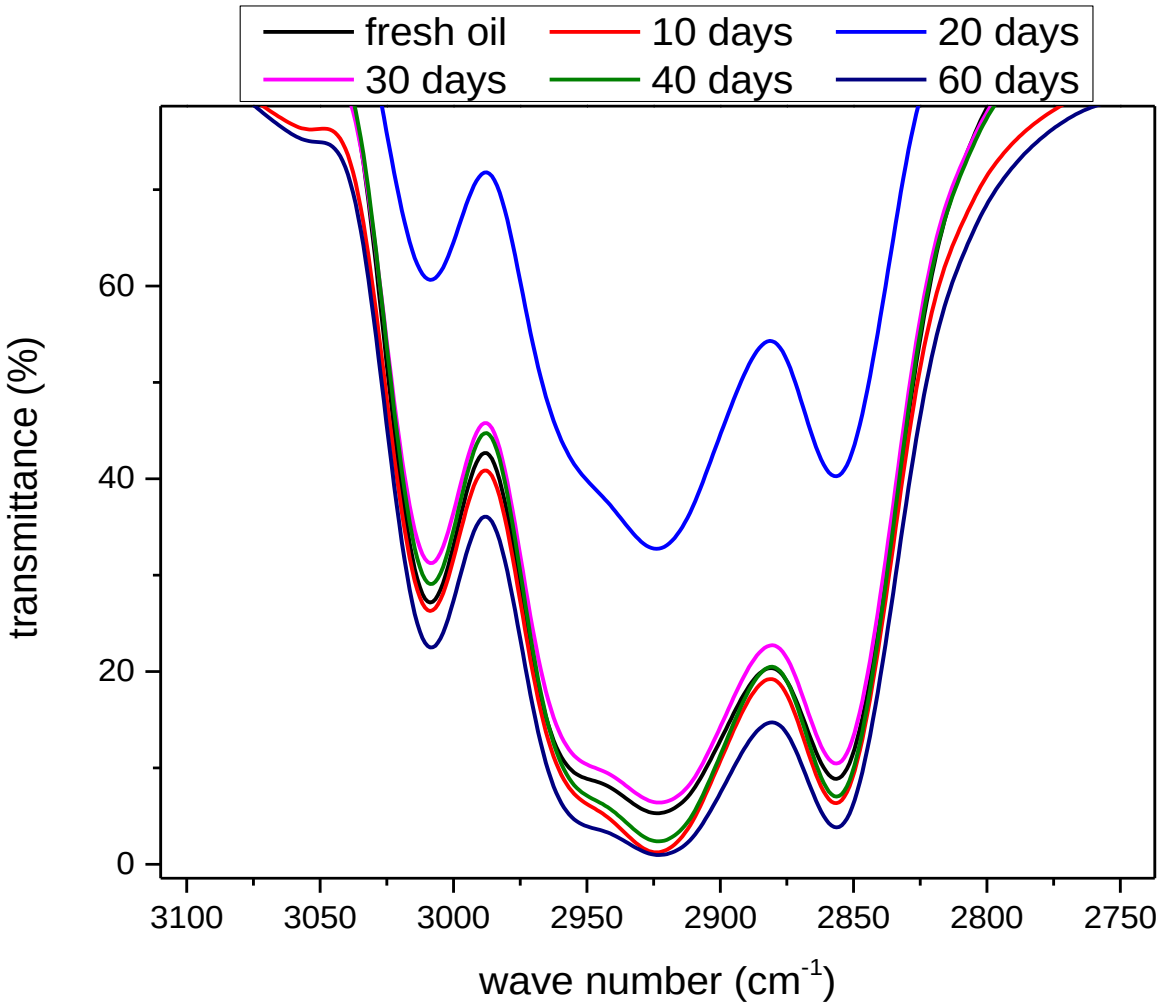



**Figure 9:** $CH_2/CH_3$ intensity evolution between 3000-2840 $cm^{-1}$ (Zoom of Figure 5)

**IV.4.1.3 the vibrational bands of the C=O and C-O:**

**Figure 10** and **Figure 11** show that after a 20-day delay, as the time of exposure to air and sun increases, the relative concentration of C=O and C-O remains almost stable.

The C=O groups at 1739 cm-1 and C-O at 1149 cm-1 show similar behavior.

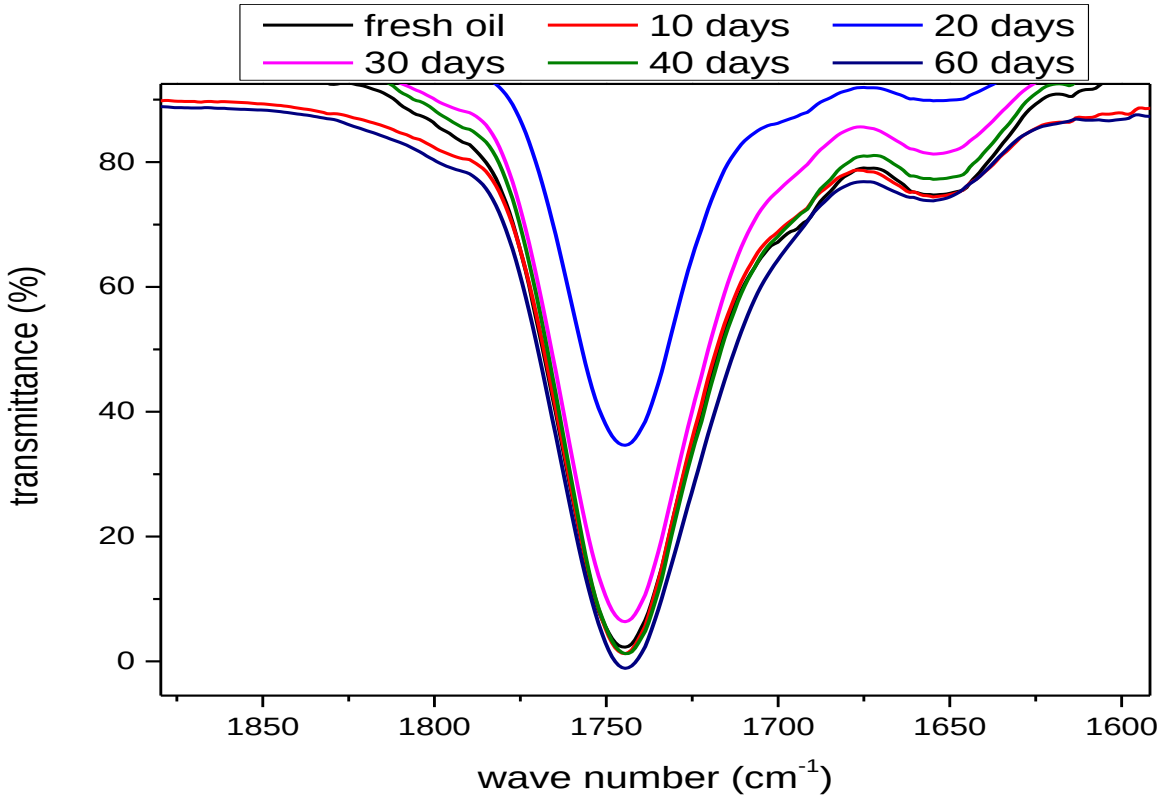



**Figure 10:** C=O band evolution 1760-1720 $cm^{-1}$ (loop of Figure 5).

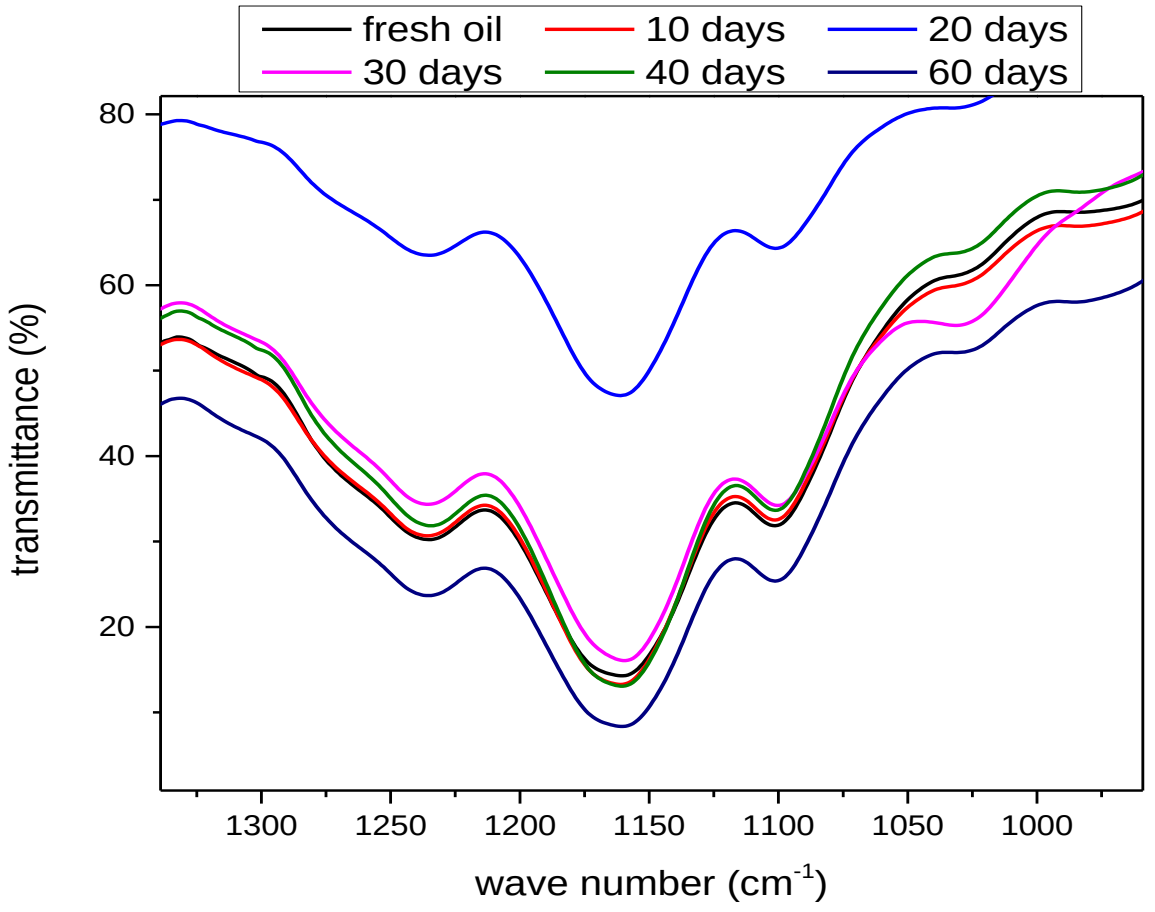



**Figure 11:** C-O-C group evolution 1200-1000 $cm^{-1}$ (loop of Figure 5).

The dryness of vegetable oils is an indication of their ability to "dry", at room temperature, in the presence of oxygen in the air. Dryness is officially defined as "the property of certain substances applied in a thin layer to evolve irreversibly from the liquid state to the solid state by oxidative polymerization under the action of air and possibly light" **[9]**. This property is due to the presence of instabilities contained in the aliphatic chains of the fatty acids that make up the oil.

In the presence of oxygen from the air, the double bonds induce radical polymerization and thus drying of the material.

The oxidation of oils has been demonstrated by the absorption of oxygen molecules. Until 1950, two hypotheses concerning the reaction of oil with oxygen coexisted **[6]**. The first hypothesis assumed saturation of the ethylenic bond by oxygen fixation, while the second involved the formation of hydroperoxides on an allylic carbon. This second hypothesis proved to be correct because it was observed that hydroperoxides were formed while the double bonds were conserved [**7]**. It appeared that the oxidation mechanism can only be explained by a radical reaction involving the removal of an allylic hydrogen atom by a free radical **[10]**.

Indeed, oils contain impurities that are easily oxidized, such as hydroperoxides formed during manufacturing processes, etc. The radicals (noted X•) resulting from the thermal decomposition of these impurities, or generated in contact with the atmosphere, can tear off a hydrogen atom from the fatty acid chain and initiate a radical oxidation reaction as shown in **Figure 12 [11-12]**.

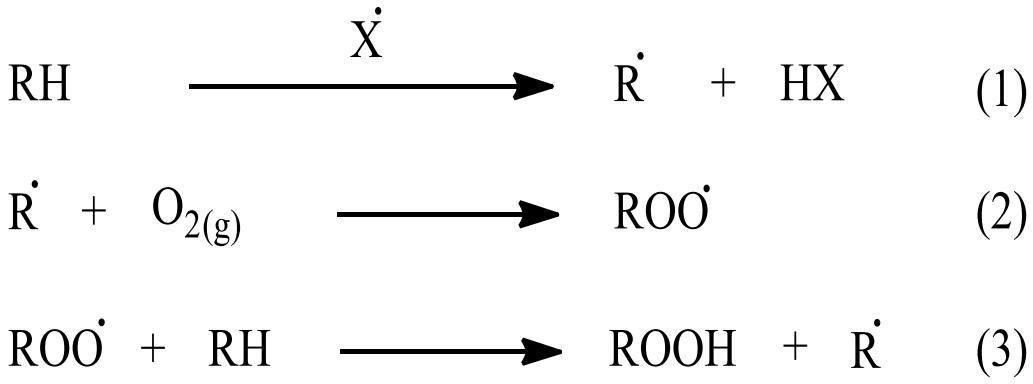



**Figure 12:** Oxidation reaction by $O_2$.

In fact, reaction (2) must be broken down into several steps. Indeed, the R• radical (in the case of methyl linoleate or any other fatty acid ester with a methylene group activated by double bonds) can evolve towards a more stable state where the double bonds are conjugated and whose configuration can be modified. This radical isomerization reaction is very rapid

and precedes the addition of oxygen to the radical. The formation of hydroperoxides can therefore be interpreted by the mechanism **[13]** represented in **Figure 13**.

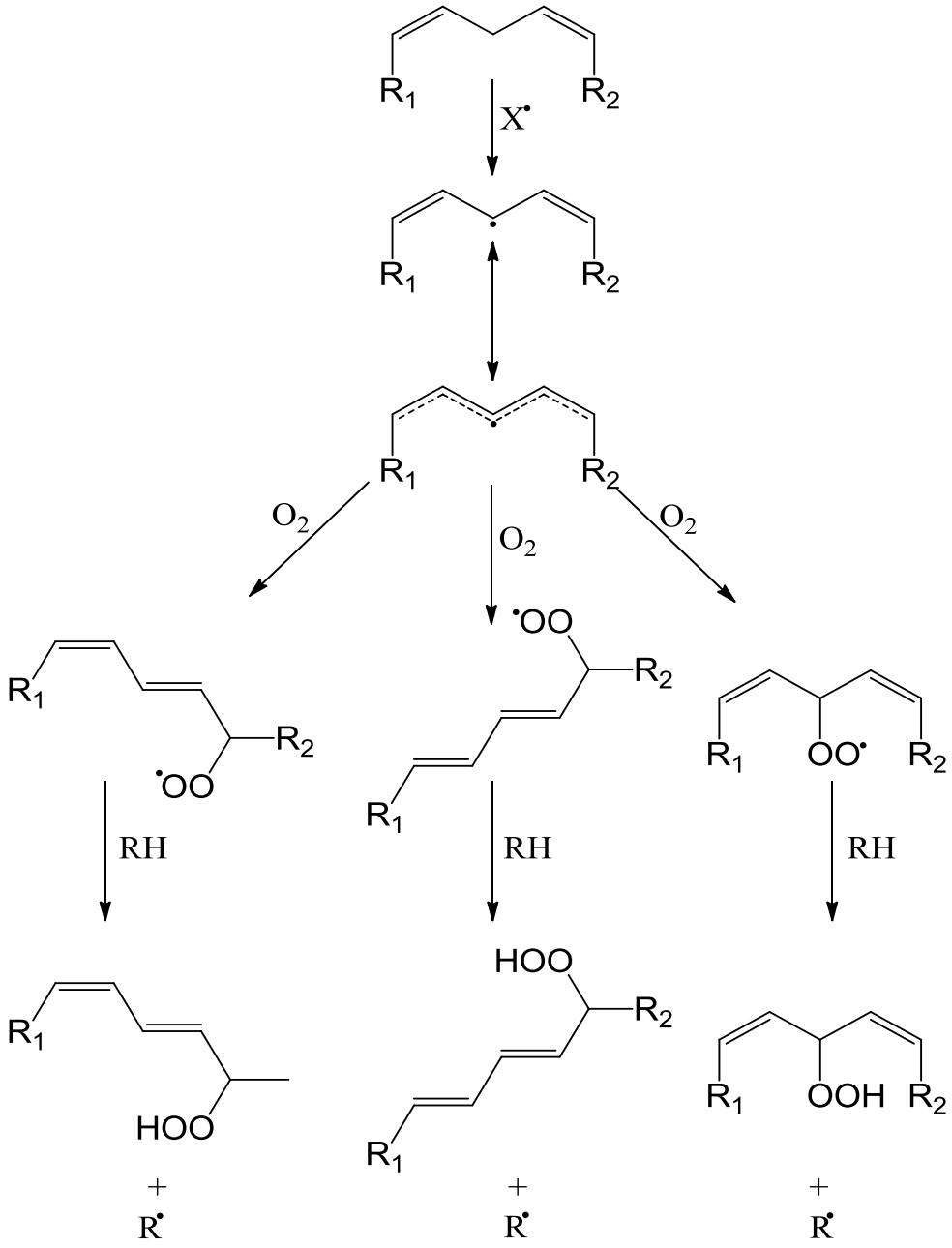



**Figure 13:** Hydroperoxides formation mechanism**.**

Our results confirm this hypothesis:

Certainly, the chemical and physical analysis as well as the follow-up by FTIR spectroscopy have well distinguished between two periods of which the first one lasts 20 days and is characterized by the formation of free fatty acids followed by the second part characterized by the formation of hydroperoxides at the starting point with regard to the length of the carbon chain and the degree of oxidation.

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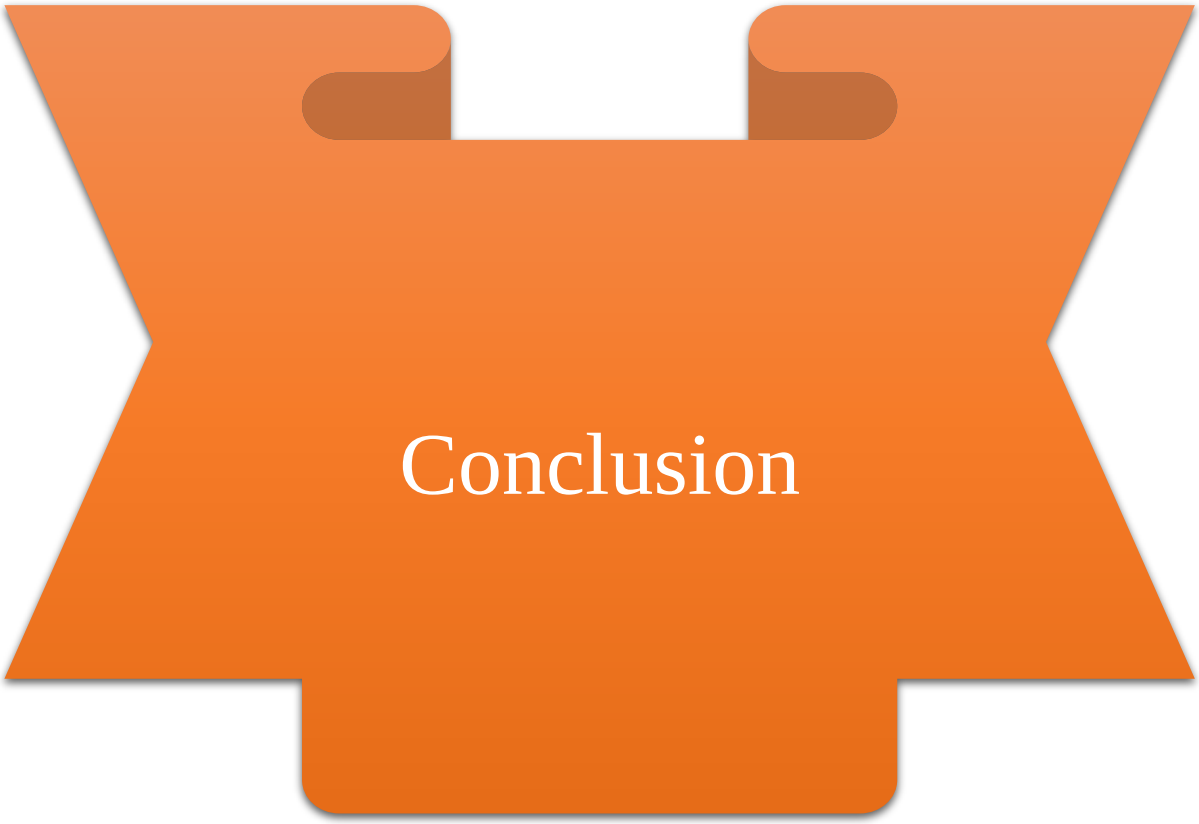
Conclusion

# Conclusion

Polyunsaturated fatty acids, such as linolenic acid, do exist in interesting proportions in flaxseed oil and their benefits, notably in terms of protection against cardiovascular diseases and cancers, have been widely studied over the last few decades.

On the other hand, these molecules are very sensitive to oxidation, which could cause premature rancidity of the oils. To protect its main molecules from oxidation, flaxseed oil has developed a defense system: phenolic compounds. These compounds are partially found in the oil, which allows it to last longer. They help protect the oil from oxidation, and it is mainly their in vivo role that scientists have been working on. Indeed, the radicals generated by oxidative stress have often been identified as the cause of the main causes of mortality in developed countries: cardiovascular disease and cancer. Their stabilization seems to be a major issue and this explains the growing interest in antioxidants. The incorporation of Gallic acid in flaxseed oil has proven its ability to stop the oxidation of this oil while inhibiting the formation of secondary compounds such as hydroperoxides and secondary products.

A second main objective of this study was to develop and explore the potential of the infrared spectral method as a rapid approach to assess the quality of an oil exposed to air.

FTIR spectroscopy showed the effect of air oxygen on the quality of vegetable oils as a function of time on the following points:

**I.** The first stage of degradation occurs in the first 20 days.

**II.** After this period, the oil shows a return to the starting point in terms of the length of the carbon chain and the degree of oxidation as well as the non-variation in the quantity of the "cis" isomer giving the impression that the second type of degradation, which is the photopolymerization that’s done without changing the double bond.

**Résumé**

Dans notre travail, on a fait appel à un antioxydant qui est l'acide gallique connu dans la littérature par son effet antioxydant très élevé .Des quantités en masse sont additionnées dans l'huile de lin sont mises sous des conditions de stockage dans l'obscurité pendant 60 jours.

Les résultats obtenus en ce qui concerne l'acidité, l'indice de peroxyde et l'absorbance ont montré l'effet très efficace de l'acide incorporé en question de stopper la détérioration de l'huile de lin en matière de formation des composés secondaires d'oxydation.

Ce travail avait, également, pour objectif d'explorer le potentiel de la spectroscopie FTIR comme approche rapide pour estimer la qualité d l'huile de lin tout en suivant la dégradation d'un échantillon exposé à l'air et soleil pendant 60 jours.

La spectroscopie FTIR a montré l'effet de l'oxygène de de l'air sur la qualité des huiles végétales en fonction du sur les points suivants :

- une diminution des longueurs des chaînes carbonées dans les huiles au cours de l'exposition à l'air et soleil. Ce phénomène est à l'origine de la diminution des intensités d'absorbances des bandes de vibration d'élongation symétrique (2854 $cm^{-1}$) et asymétrique (2924 $cm^{-1}$) et de la bande de déformation du groupe $CH_2$ vers de 724 $cm^{-1}$.
- une augmentation du degré d'oxydation de l'huile (visible au niveau des groupes C=O et C–O).
- stabilité de la quantité de l'isomère "*cis*" lors de la formation des peroxydes.

**Mots clés :** huile de lin, antioxydants, acide gallique, spectroscopie FTIR.

**Abstract**

In our work, we used an antioxidant, which is Gallic acid known in the literature by its very high antioxidant effect. Mass quantities are added in flaxseed oil and saved in dark storage conditions for 60 days.

The results obtained regarding acidity, peroxide value and absorbance showed the very effective effect of the incorporated acid in question to stop the deterioration of linseed oil in terms of formation of secondary oxidation compounds.

This work also aimed to explore the potential of FTIR spectroscopy as a rapid approach to estimate the quality of linseed oil while monitoring the degradation of a sample exposed to air and sun for 60 days.

FTIR spectroscopy showed the effect of air oxygen on the quality of vegetable oils in the following points:

- A decrease in the length of carbon chains in oils during the exposure to air and sun. This phenomenon is at the origin of the decrease of the intensities of absorptions of the bands of vibration of symmetrical elongation (2854 $cm^{-1}$) and asymmetrical (2924 $cm^{-1}$) and of the band of deformation of the $CH_2$ group towards 724 $cm^{-1}$.
- An increase in the degree of oxidation of the oil (visible in the C=O and C-O groups).
- Stability of the quantity of the "cis" isomer during the formation of peroxides.

**Keywords**: flaxseed oil, antioxidants, Gallic acid, FTIR spectroscopy.

**خلاصة:**

في عملنا قمنا باستخدام مضاد أكسدة يسمى بحمض الغاليك، والمعروف مرجعياً بتأثيره القوي كمضاد أكسدة، تمت إضافة كميات مختلفة منه إلى زيت بذور الكتان بشكل منفصل وتخزين هذه الأخيرة في الظلام لمدة 60 يوم.

النتائج المتحصل عليها والمتعلقة بالحامضية، مقدار البيروكسيد والامتصاصية أظهرت التأثير الفعال للحمض المستخدم لأجل إيقاف تدهور حالة زيت بذور الكتان وتشكل مواد التأكسد الثانوية.

هذا العمل يهدف أيضاً إلى اكتشاف إمكانيات مطيافية الأشعة ما تحت الحمراء بتحويل فوريي FTIR كمقاربة سريعة لتقييم جودة زيت بذور الكتان أثناء مراقبة تحلل عينة الزيت المعرضة للهواء لمدة 60 يوم.

مطيافية الأشعة ما تحت الحمراء بتحويل فوريي FTIR أظهرت تأثير أكسجين الهواء على جودة الزيوت النباتية فيما يلي:

- تقلص طول السلاسل الكربونية بالزيوت أثناء التعرض للهواء وضوء الشمس، هذه الظاهرة تتجلى في انخفاض شدة الامتصاص لكل من قمة اهتزاز التمدد المتناظر ($2854\ cm^{-1}$) والغير متناظر ($2924\ cm^{-1}$)، وقمة اهتزاز الزاوي لمجموعة $CH_2$ حوالي $724\ cm^{-1}$.
- زيادة درجة تأكسد الزيت (تأكسد واضح عند المجموعات C=O وC-O).
- استقرار كمية الإيزومير 'cis' أثناء تشكل البيروكسيدات.

**الكلمات المفتاحية**: زيت بذور الكتان، مضادات أكسدة، حمض الغاليك، مطيافية الأشعة ما تحت الحمراء بتحويل فوريي FTIR.