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Protection of olive oil from degradation

Jury :

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DEDICATION

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

I wille dedicate this work

To my dear parents, For their patience, love, support and encouragement

To my brothers :Abdelake and Mostafa

Also my lovely sisters : Minat allah , Sara and Dikra

To our family friend :Uncle Labed Lakhdar

To my best friends :Rahma, Rania, Faiza , Chaima and Rima sara

and all my comrades

Not to mention all the teachers, whether primary, middle, secondary

Or from higher education.

Rima bentayeb

Dedication

I dedicate this modest work

- *To my father, my childhood school, who was my shadow during all the years of study, and who has made sure throughout my life to encourage me, to give help and protect me.*

- *To the one who gave me life, the symbol of tenderness, who sacrificed himself for my happiness and my success, to my mother*

May GOD guard and protect them.

- *To my brother Mounir and my adorable sisters: Fida , Mouna*
- *To my adorable sisters, their husbands and their children: Aya, Ritel , Ziad , Sadjed , Shahed and Afnan*
- *To my dear colleagues and my friends: Rima , Rayan , Mouad …*

You are to me brothers, sisters and friends on whom I can count.

As a testament to the friendship that unites us and the memories of all the moments we spent together, I will dedicate this work and I wish you a life full of health and of happiness.

LEBBal MERIEM

Thanks

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List of abreviations:

A :	Acidity
A (λ) :	Absorbance at the wavelength λ
AFMI :	Mono unsaturatedfattyacids.
AFPI	Polyunsaturated fattyacids
IA :	Acid Index
IP :	Peroxide Index
C.O.I:	International Olive Council
K_{232} :	Specific extinction coefficient at 232 nm
K_{270} :	Specific extinction coefficient at 270 nm
Meq:	milliequivalent.
Ppm :	Part per million
KOH :	Potassium hydroxide
$Na_2S_2O_3$:	sodium thiosulfate
NaOH :	Sodium hydroxide
UV :	ultra-violet
pH :	potential of hydrogen
AW :	Water activity

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General introduction

The olive tree is an emblematic plant of the Mediterranean. Indeed, it is no wonder that most of the world area dedicated to this crop is found, in fact, in the Mediterranean Basin. This is where 95% of production and 85% of world consumption **[1].**

The nutritional, biological, taste, and physicochemical properties of the olive oil explain consumer interest in this oil, which is recognized as a essential component of the Mediterranean diet **[2].**

Olive oil is made up of around 99% triglycerides. Its acid composition fat is characterized by a high content of monounsaturated fatty acids, particularly in oleic acid and polyunsaturated fatty acids (linoleic, and linoleic acid).

Besides this interesting background, the other attraction of olive oil lies in its richness in minority compounds such as polyphenols. The nutritional value of these compounds phenolics lies in their strong antioxidant capacity which could prevent or slow down the appearance of certain degenerative diseases as well as cardiovascular diseases **[3].**

Polyphenols are of great importance for the stability of virgin olive oil compared to other refined oils where these are eliminated during refining process **[4]**. Major phenolic compounds in olive oils virgin are tyrosol and hydroxytyrosol, followed by traces of substituted cinnamic acids including caffeic acid, oleuropeilaglycone and oleuropein **[5].**

This resistance to oxidation can be improved by several methods. One of the most appropriate approaches are the incorporation of antioxidants which consists of delaying oxidation of the oil and prolong its durability. This can be achieved by incorporating vegetable plants (rosemary) containing natural molecules with activity antioxidant in olive oil mass. It is within this framework that our work falls

which is distributed as follows:

Chapter I: Studies of vegetable oils.

Chapter II: Study of the oxidation of vegetable oils.

Chapter III (first part): Materials and Methods.

Chapter III (second part): Discussion of the results

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Chapter I:

Study of vegetable Oils.

Chapter I : Vegetable oils

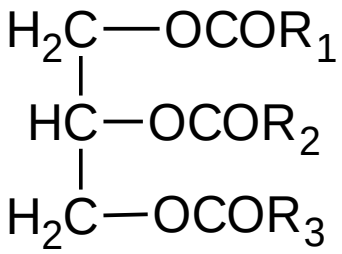
I.1.History:

Oils have been used by humans since ancient times and their uses have evolved over the centuries. The first pressed oils were probably oil from sesame and olive oil, the olive tree being cultivated in the Mediterranean 6000 years ago.

The first use of the oil had no food vocation but rather fuel for lighting **[1]**.

I.2 Definition:

In general, the word "oil" refers to the triglycerides that are found in their state.liquid at room temperature. They are found in several plants includinglegumes (peanuts, soybeans), seeds (rapeseed, sunflower), fruits (almond, olive, palm, grape seeds), cereals (corn) or in cotton. Their formula general is written:



Vegetable oils are non-volatile, hydrophobic and sometimes organic compound samphiphiles. They are insoluble in water, soluble in non-polar organic solvents and are part of the natural constitution of certain plants, whether cultivated or not.

A vegetable oil is extracted from the plant by cold pressing from two organs main, seeds and fruits. Plants rich in oil are called oilseed or oilseed plant [**2]**.

Vegetable oils are usually subdivided into two main classes:

Fluid vegetable oils :peanut, rapeseed, corn germ, sun flower,soy and olive.

Concrete vegetable oils (fat):copra (from coconut), coconut oil palm **[3]**.

Vegetable oils are privileged sources of essential macronutrients name lylinoleic acid (omega 6 family) and alpha-linoleic acid (omega 3 family)and micronutrients (vitamin E, phytosterols) **[4]**.

I.3 Chemical composition of vegetable oils:

Vegetable oils are made up of a wide variety of constituents (Figure 01) and their chemical compositions are represented by fractions called fractions saponifiable (98-99%) and unsaponifiable (1-2%).

Triglycerides are in the majority and represent at least 95% of the weight of crude oils and 98% by weight of refined oils. Other constituents naturally present in smaller quantities, are said to be minor constituents (1 to 5%) and group together compounds with varying structures such as phospholipids (0.1-0.2%), sterols ,tocopherols (vitamin E) and some anti-nutrients **[5]**.

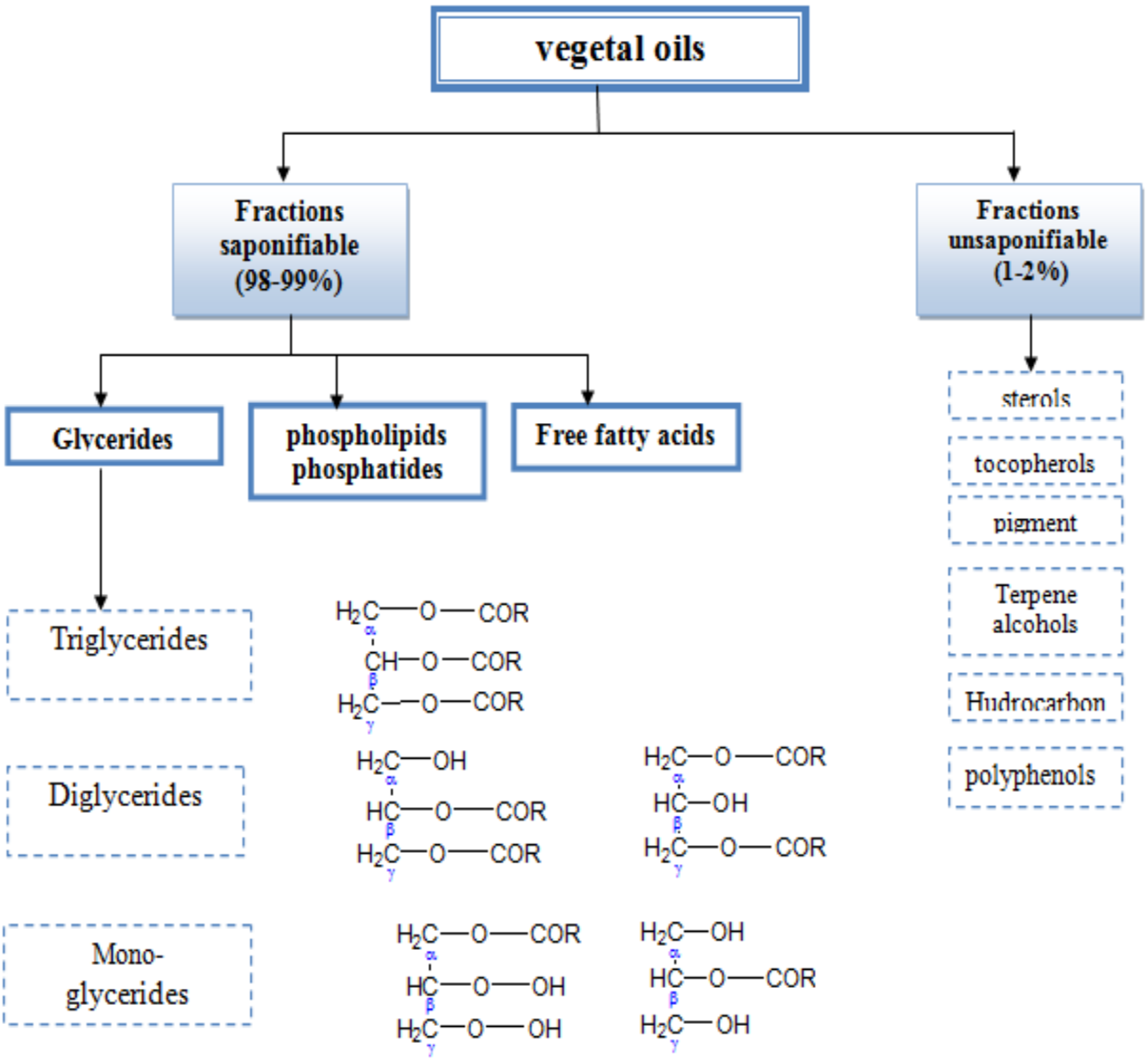


Figure 01: Constituents of vegetable oils.

I.3.1 saponifiable fraction:

This fraction is made up of two constituents, triglycerides and fatty acids.

A) Triglycerides:

Structurally, a triglyceride is made up of a glycerol molecule combined with three molecules of fatty acids. Glycerol is made up of a chain of three carbon atoms each

comprising a hydroxyl group (-OH). These groups hydroxyls react with the carboxyl group (-COOH) of fatty acids to form esters **[6]**.

When a glycerol molecule is linked to three molecules of the same acid, the triglyceride formed is said to be homogeneous or monoacid. Otherwise, the triglyceride is said to be mixed **[6].**

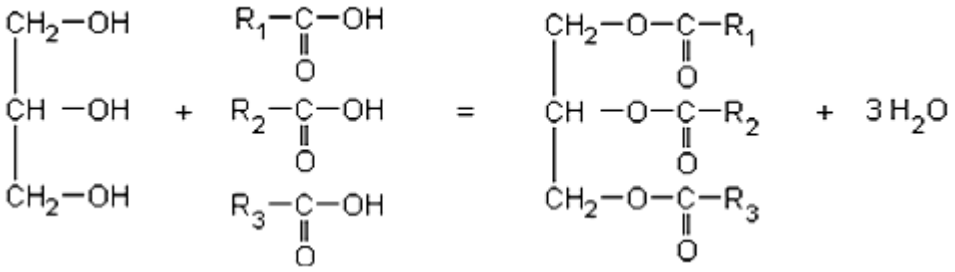


Figure 02: Fatty acid triglycerides.

B) Fatty acids:

Fatty acids are organic compounds with the general structure R-COOH, made up exclusively carbon (C), hydrogen (H) and oxygen. The weight of a molecule of fatty acid is distributed among these three elements in the respective proportions of 76%,12.7% and 11.3% **[6]**.

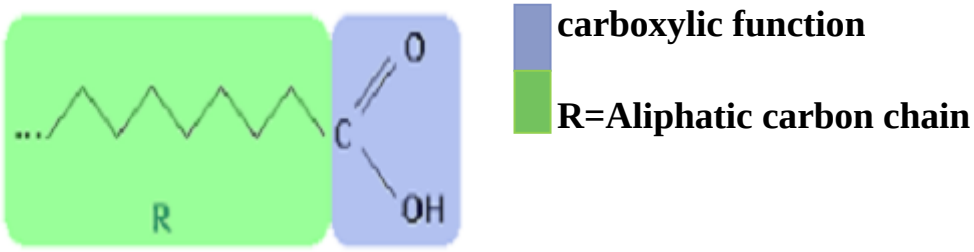


Figure 03: Structure of fatty acids.

Several different fatty acids may be present in the same fatty body and, one fatty (oleic acid for example) can be found in many different fats. The fatty acid composition is often characteristic of their sources, particularly Plant.

However, there are variations in climate origin or related to seasons, physiological status and diet **[7]**.

Fatty acids differ between them by:

- The length of the carbon chain.
- The number, position and spatial structure (cis, trans) of the double links **[8]**.

Fatty acids can therefore be categorized into three groups:

❖ **Saturated fatty acids:**

They are made up of a hydrocarbon chain not containing double bonds and have for general formula CH_3 - (CH_2) n - COOH. They are strong and fairly stable at ambient temperature.

The most common are: palmitic acid (C16: 0) and stearic acid (C18: 0) **[7].**

❖ **Unsaturated fatty acids:**

They are fluid at room temperature and are classified into two categories:

➢ **Monounsaturated fatty acids:**

We speak of monounsaturated fatty acid when there is only one double bond.

Monounsaturated fatty acids have the following chemical formula:

$$H_3C - (CH_2)\ n - HC = CH - (CH_2)\ p - COOH$$

Where: n and p are positive or zero integers.

Example: Oleic acid: (18: 1n-9) or (ω9).

➢ **Polyunsaturated fatty acids:**

These are acids that contain several instigations. There are two families of acids essential polyunsaturated fat named n-3 (famille3 family) and n-6 (ω6 family) compared to at the position of the last double bonds. Two fatty acids are at the origin of these families; it is linoleic acid (C18: 2 n-6), precursor of omega 6 and α– acid linoleic (C18: 3 n-3), precursor of the omega 3 family **[9]**.

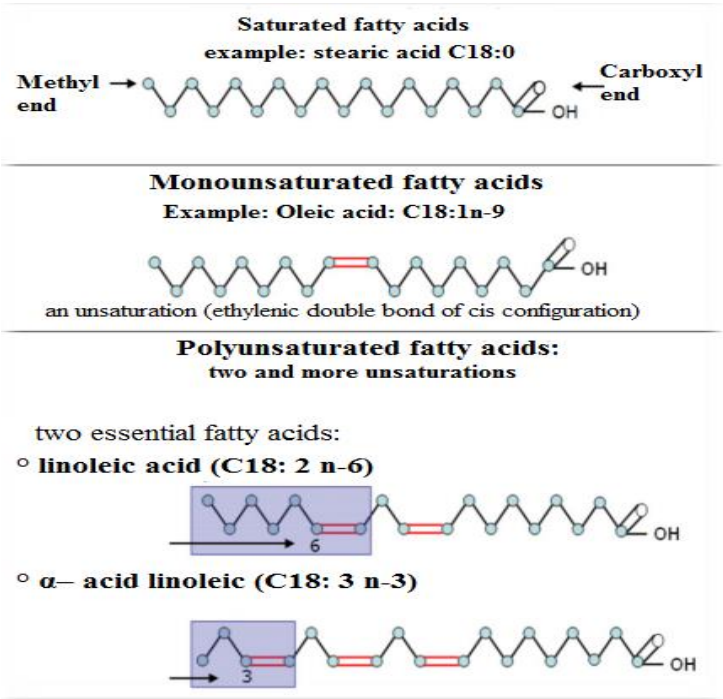


Figure 04: Structure and nomenclature of the main fatty acid families [10].

I.3.2 unsaponifiable fraction

Vegetable oils also contain non-glyceridic constituents and lipids complexes called "minor constituents". The content of these constituents is very low.

I.3.2.1. Fat-soluble vitamins:

- **Vitamin E (tocopherol):**

Vitamin E, also known as tocopherol, is found in significant quantities in oils plant and essentially has antioxidant properties which participate in the preservation of oils. It comes in four forms (α, β, γ, δ). Its content varies from200 to 1200 mg / kg in vegetable oils.

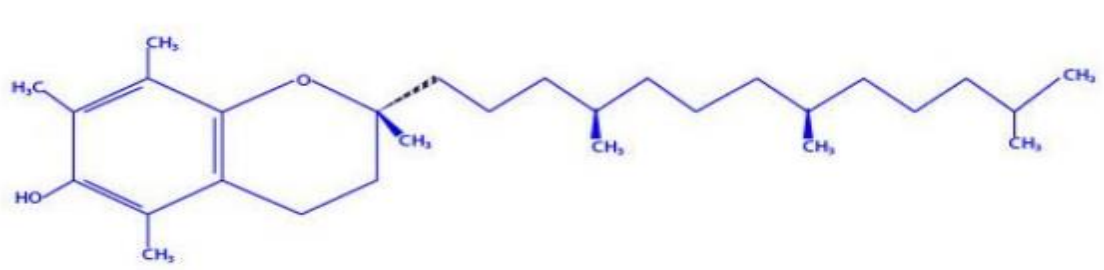


Figure 05: Structure of vitamin E [11].

- **Vitamin A and carotenoids**

Carotenoids are highly unsaturated hydrocarbons, ranging in color from yellow to orange **[12]**. They are liposoluble pigments sensitive to heat, light
and ultraviolet rays and having the empirical formula C40H56. They include carotenes and xanthophylls and come in several types (α, β, γ,). The main ones carotenes found in vegetable oils are β carotenes **[12]**.

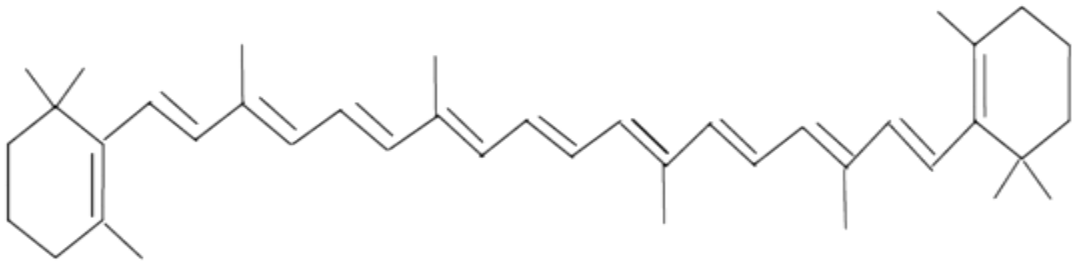


Figure 06: Structure of carotenoids [13].

I.3.2.2 Antioxidants

- **Tocotrienol:**

It is a structural analogue of tocopherol which has certain properties physiologic not seen in tocopherol; for example his activity cholesterol lowering **[14].**

- **Phytosterol:**

All vegetable oils contain it (from 0.1 to 0.5% on average) and their structure molecular has strong analogies with cholesterol. Provided in sufficient quality through food (2 to 3 g / d), they have a cholesterol-lowering role which cannot be obtained only with the consumption of products enriched with phytotrons since the total intake daily is estimated to be less than 500mg **[15]**.

I.4 Characteristics of vegetable oils:

I.4.1. Physical properties:

I.4.1.1. melting point:

It allows you to appreciate the degree of purity of a fatty substance. It depends on the degree of establishment and the length of the carbon chain.

In unsaturated fatty acids, the melting point increases with the length of the chain hydrocarbon.

Unsaturated fatty acids have a lower melting point than saturated fatty acids**[16].**

I.4.1.2.Density:

Density is the ratio of the mass of a certain volume of oil to the mass of the same volume of distilled water. It must always be less than 1 **[17]**.

I.4.1.3 solubility:

All fatty acids with a carbon number greater than 8 are insoluble in water and generally soluble in organic solvents such as ether, chloroform and benzene **[16]**.

I.4.1.4.Viscosity:

The viscosity of fatty acids and triglycerides is related to their structures (length of the chain and saturation). It increases with molecular weight and decreases with increasing the number of insturation (double bonds) and temperature.

I.4.2 Chemical properties:

I.4.2.1.Hydrolysis and saponification:

Hydrolysis of fatty substances leads to the release of one or more fatty acids providing glycerol and a mixture of carboxylates (sodium and potassium). The reaction can be carried out with sulfuric acid or enzymatically.

There are two types of hydrolysis reactions:

- **Enzymatic hydrolysis:**it affects only crude oils.
- **Spontaneous hydrolysis:**it occurs during storage and processing thermal.

Saponification is a reaction which allows the transformation of free fatty acids or combined in soap in the presence of potassium or soda (KOH or NaOH) **[18]**.

1.4.2.2. Hydrogenation:

The hydrogenation of unsaturated fatty acids is done using hydrogen (H_2) in the presence catalysts, under high pressure (100 to 200 bar) and temperature varying from 200 at 400 ° C **[19]**.

I.5 Methods of extracting vegetable oils:

The production of vegetable oils has always been the main objective of the cultivation of seeds. Extraction methods have evolved but the process of extracting oils always remains the same. Generally there are four methods which are based on either expression or volatility.

I.5.1. Classic or traditional process:

In classic (traditional) extraction units, the oil extraction process consists of the following different steps:

I.5.1.1. Grinding:

It is carried out by granite stone grindstones, which turn in a tank whose soil is also in stone. This grinding is carried out manually or by means of a animal. This step therefore makes it possible to obtain a paste which contains solid matter (debris nuclei, epidermis, cell walls, etc.) and fluids (oil and water from vegetation, i.e. the water contained in the cells of the seed).

I.5.1.2 Phase separation:

The dough produced is put on scourtins (vegetable fiber discs). Then a oil extraction is carried out by pressure. Pressing generates a by-product solid called pomace. These pomace are the solid residues recovered following the first pressing or centrifugation. They are made up of the residues of the skin, pulp, and fragments of kernels.

A separation by settling of the liquid phases (oil and vegetation water) is performed. This separation is done in the open air in cement, earthenware or clay.

A liquid by-product was generated at the end of this step, called vegetable waters. It's the brown aqueous liquid residue which separated from the oil by sedimentation after pressing or centrifugation. This liquid has a pleasant smell but a bitter taste. This effluent relatively rich in organic matter constitutes a pollution factor which creates areal problem in the olive industry.

I.5.2 Batch process or super press system:

The seeds received in traditional oil mills go directly through the following steps:

I.5.2.1. Grinding:

It is carried out by millstones. The grinding wheels used for grinding are slightlyoff-center with respect to the axis of rotation, which increases the possibility of crushing seeds.

I.5.2.2.Mixing:

This step frees as much oil as possible. Raclettes constantly bring back the dough under the grindstones which then play the role of kneaders. The dough is obtained at the end about half an hour.

Figure 7: Mixing the dough.

I.5.2.3. Phase separation:

The paste is then placed in a layer approximately 2 cm thick on fiber discs of nylon (the scourtins), themselves stacked on top of each other around a central pivot (called needle) mounted on a small carriage. The assembly is placed on a press piston hydraulic system which allows the dough to be subjected to a pressure of around 100 $kg.Cm^{-2}$.

The liquid phase flows into a tank. The pomace remains on the scourtins. This operation takes about 45 minutes. Then, each scourtin is free of its pomaceby tapping it as when cleaning a carpet.

Figure 8: Dough placed on scourtins.

I.5.2.4. Settling:

Oil, having a lower density than water, so it rises to the surface. He this is natural settling. However this method is hardly used any more, in due to its slowness and the difficulty in separating oil from water in the vicinity of the interface between the two fluids. These are vertical plate centrifuges that now make it possible to separate oil from vegetable waters.

I.5.3 Continuous process:

There are two types of the continuous extraction process: three centrifuge system phase and two-phase centrifugation system.

I.5.3.1 Three-phase centrifugal extraction system:

The seeds, once received, undergo preliminary treatments such as stripping, destining (removing stones) and washing in order to have good quality.

I.5.3.1.1. Grinding:

It is carried out by mechanical disc or hammer mills. These crushers can work continuously, the dough being obtained almost instantly.

I.5.3.1.2.Mixing:

The dough is poured into a stainless steel tank moderately thinned with lukewarm water, in which turns a spiral or worm, also in stainless steel.

I.5.3.1.3. Phase separation:

It consists in separating the solid part (pomace) from the fluid part (vegetable waters). Dough mixed is injected by a pump into a centrifuge whose axis is horizontal(horizontal settling tank)

I.5.3.1.4. Settling:

Vertical plate centrifuges are used to separate the oil from the vegetable waters.

I.5.3.2 Two-phase centrifugal extraction system:

The grains undergo the same stripping, stone removal, washing and grinding stages, of mixing and settling than those of the previous three-phase system. However, this present grain extraction process works with a new decanter withtwo-phase centrifugation which does not require the addition of water for the separation of oily and solid phases containing pomace and vegetable waters.

This two-phase decanter achieves slightly higher oil yields. higher than those obtained by the conventional three-phase decanter and the hurry. In addition, it does not increase the volume of vegetable waters.

I.5.4 Solvent extraction:

Solvent extraction of oils and grease is mainly carried out industrially by contacting the oleaginous material to be treated with a suitable solvent. We obtain thus an oil solution in the solvent or mixture, the concentration of which varies according to the quality of the solvent and the oil content of the treated oilseed.

This mixture, after filtration and concentration, is subjected to the action of heat in aevaporator then in a vacuum finishing column comprising a vapor injection for the total elimination of the last traces of solvent. The vapors thus produced are condensed in conventional devices and the oil obtained is cooled and stored in waiting for refining.

The condensed solvent is collected in a separator where it is freed from the water coming from the injected steam and the humidity of the treated products.

Extraction flours always retain a fairly large quantity of solvent, which it is recovered in drying devices. Here too, the last traces of solvent are driven out with live steam or under more or less high vacuum.

The extraction is carried out by a soxhlet apparatus or a Lickens -Nickersen apparatus.

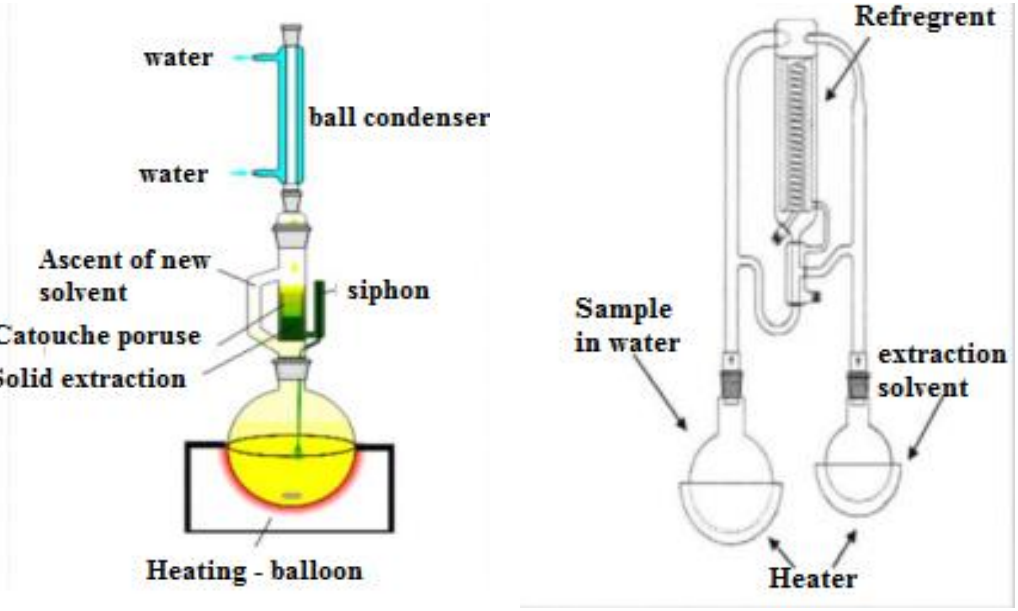


Figure 9: Soxhlet apparatus on the left, and Lickens-Nickerson apparatus on the right.

Among the solvents that can be used, we can cite several types: petroleum derivatives, chlorinated solvents and alcohols.

I.6 role of vegetable oils:

Vegetable oils can contribute significantly, depending on their composition in fatty acids, to improve the overall balance of the lipid content of a diet. They like fats in general, fulfill four main roles:

- **nutritional (energy and nutrient supply):** fatty acids, vitamins fat-soluble, minor constituents of interest such as phyto sterols,
- **organoleptic:** flavor and aroma carrier,
- **rheological:** texture,
- **technological:** heat transfer fluid, for example in frying applications **[19].**

It should be noted that only a third of the world production of fatty substances is intended for industrial use. Two thirds of production is in fact intended for food.

Among the many industrial uses of fatty substances, we can cite the manufacture of soaps and cosmetics, fatty acids, etc. Triglycerides are also the source of many chemicals that can go into the composition of a multitude of products: lubricants, cosmetics, pharmaceuticals, paints, etc... **[20].**

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Chapter II:

Study of vegetable oil oxidation

Chapter II: Study of vegetable oil oxidation

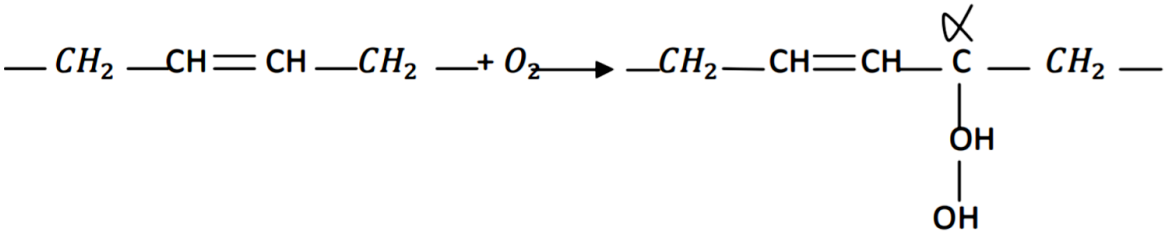
II.1.Introduction:

Oxidation can occur during the processing of oils from raw materials to food storage, as well as during storage, consumption and use. The form in which the oils are presented is an important element, namely in free form, of tissue fat or confined to a tissue structure or even in a dispersed phase.

II.2.Dosage of oxidation products [1]:

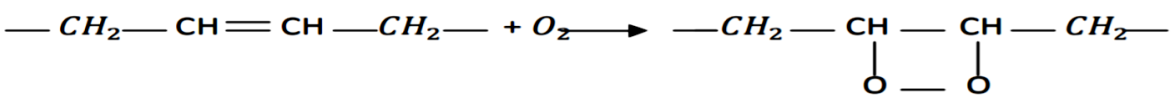
Oxidation is a fundamental phenomenon in all fat industries: oil, margarine, soap, cosmetic, livestock feed the chemical alteration of fats unsaturated by oxygen from the air leads to the formation of a peroxide . This reaction being a self catalytic reaction, begins slowly and then after a period of induction or oxidation is virtually undetectable accelerates exponentially.

Oxygen attaches to fat chains depending on the temperature at which oxidation takes place: below 60%, the predominant reaction is the formation of a hydroperoxide in α of the double bond



Oxygen fixation is accompanied by a shift in the double bond that leads to polyunsaturated acids more easily oxidable than monounsaturated acids to the formation of dienic systems conjugated mostly in trans form.

Between 60 and 130 degrees, the mode of formation of peroxide is different because the oxygen attaches to the double lais to give an epiperoxide



The «split products» resulting from the cut of the fat chain at the double bond, which yields a range of short chain compounds

more important are : adehydes and ketones. These adehhydes can themselves peroxide and

then degrade into an adehhyde to one less carbon atom than primitive adehhyde and formic acid.

It is the split products that are responsible for the smell of rancid fats.

Oxidized products of the same chain length as the starting acid of unsaturated ketones, «dicetones» and hydroxy acids that make up the majority of the 'oxidized acids'. Under certain influences, especially that of the H ions, the unsaturated peroxides decompose in a very particular way to give an unsaturated acid having a double bond more than the starting acid all the double bonds of the new compound formed being conjugated.

The ions H+ are not the only cause of this decomposition and are also observed in fatty bodies that have undergone centrifugations accompanied by accidental oxidation The oxidation products of higher molecular weight than those of the departure resulting from the polymerization of two or more oxidized chains linked together either by oxygen bridges or by carbon bonds.

Combined dienic and trienic systems appear to give rise more easily to polymerization products than to volatile products of their oxidation.

To assess the oxidation state of an oil one determines one peroxide index: There are many methods of measuring a peroxide index (IP). There are also, which is a serious source of confusion, many modes of IP expression. Currently, the three most frequently used modes of expression therefore define the IP:

- As the number of micrograms of active oxygen per gram of fat,
- As the number per millimeter moles of peroxide per kilo gram of fat,
- Like the number of mill is equivalent of active oxygen per kilogram of fat.

In a molecule of peroxide (whether hydroperoxide or epiperoxide there is an attached oxygen molecule. But of the two oxygen atoms only one is "active", that is - say analytically capable of oxidizing For example iodides to iodine.

The two most commonly used methods are iodometric methods, one that is fastest and easiest to execute, the other cold which is the internationally standardized method.

The principle is therefore the same: Iodine oxidation oxidation by the active oxygen of peroxide.

II.3.Lipid substrates exposed to oxidation phenomenon [1]:

The lipid substrates of these reactions are mainly unsaturated fatty acids, free, they generally oxidize faster than when they are part of triglycerides or phospholipid molecules .But it is above all the degree of establishment that influences the rate of

oxidation: at 100oC the relative velocities of oxidation of stearic acids (C18:2 and Linoleic C18:3).

Unsaturated and saturated fatty acids oxidize only at 60oC, while polyunsaturated fatty acids oxidize even when food is stored in the frozen state.

The main problems posed by these lipid oxidation reactions are the formation of volatile compounds of unpleasant odour, which can limit the shelf life of various foods.

The main unsaturated substrates of oxidation are often unsaturated phospholipids, licitines.

Other unsaturated substrates may experience similar oxidation reactions: some hydrocarbons found in oils, particularly squalene ($C_{30}H_{50}$) vitamin A and carotenoid and chlorophyll pigments vitamin E (tocopherol) and triterpenic alcohols.

The compounds formed peroxide are initially hydropensides and then by decomposition they form volatile or non-volatile secondary compounds (adehydes, ketones) of unpleasant flavor that depreciates the organoleptic, nutritional and sensory qualities.

that influences the rate of oxidation, the main problem posed by the

II.4.Oxydation of olive oil:

II.4.1.Compounds exposed to oxidation:

The substances of these reactions are mainly free unsaturated fatty acids. But above all the degree of introduction oxidation reactions of the oil lies in the formation of volatile compounds of unpleasant smell, which can limit the shelf life of food.

Three groups of lipid reactions can be distinguished in lipid oxidation (Figure1).

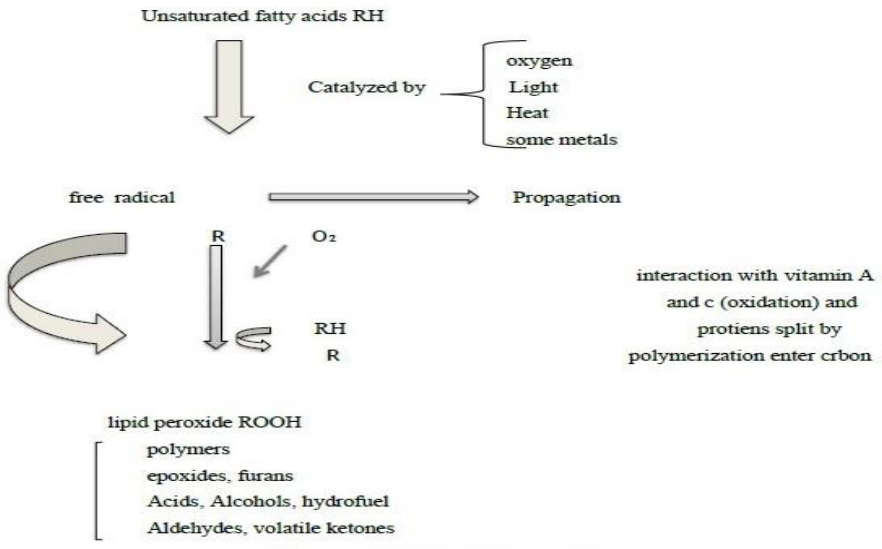


Figure 1 : Lipid oxidation reactions

Assessing the oxidative stability of oils can meet several objectives such as :

- evaluation of the effectiveness of antioxidants.
- fat resistance to oxidation
- Compliance with a specification
- Determining the sustainability of a fat substanc

II.4.2.Reaction mechanism of self-oxidation [1]:

Self-oxidation of fat abandoned on contact with oxygen is a complex set of unsolved reactions. They lead to the breakage of carbon chains with the development of products by the most volatile carboxyled structure. The organoleptic properties are altered: it is rancidity.

The self-oxidation of unsaturated fatty acids (HR) is caused by a set of chain reactions involving mostly free radicals { **R**˙}.A sequence involving an initiation stage corresponding to the activation of the fatty acid molecule, a phase of propagation and determined reactions.

II.4.2.1.Initiation[1]:

The initiation of the reaction consists of the formation of a free radical by tearing a hydrogen atom from a generally unsaturated fatty acid chain:

$$RH \longrightarrow R^{\cdot} + H^{\cdot} \quad , \quad RH + \tfrac{1}{2} O_2 \longrightarrow R + OH$$

The oxidation of the oils is at first very slow due to the low speed of initiation.

Indeed the departure of the hydrogen atom is unlikely because of the high activation energy of the reaction. However, it is facilitated by:

-heating (thermolysis)

-light (photolysis)

-ionizing radiation

When the hydrogen atom is torn off in α of the double bond, the single electron of the radical structure is stabilized by resonance.

In the case of oleic acid, the radical is formed in position n-7 or n-10.

Due to the relocation of the single electron by resonance, four free fatty acid radicals, positions isomers obtained Figure 2.

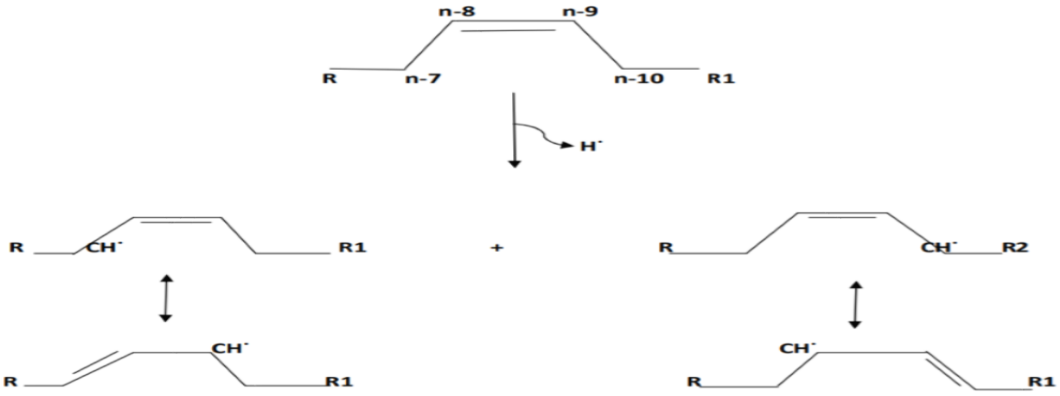


Figure 2 : Mechanism of self-oxidation of monounsaturated fatty acids.

fatty acid radicals in position n-7 or n-10 are slightly more abundant than fatty acid radicals in the n-8 or n-9 position.

However, the relative proportion of each of the free fatty acid radicals varies according to temperature. Its increase tends to homogenize the populations ofRadical.

II.4.2.2.Propagation[1]:

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \longrightarrow ROOH \text{ (hydroperoxides)} + R\cdot$$

The fatty acid radicals thus forme dreact with tripled oxygen 3O 2 dissolved in the lipid or atmospheric phase after diffusion. The reaction of a free radical with an oxygen molecule is very fast when the oxygen content is not limiting.

the interaction leads to the formation of a radical peroxy (ROO·).

The latter stabilize sits structure by tearing a hydrogen atom onto another chain of fatty acid (HR). The free radical of fatty acid (R˚) thus forme dcan continue the reaction according to the same principle, itis the propagation phase.

It is possible with out external input because the potential for oxydo-reduction of hydroperoxides (ROO·/ROOH)-1V is higher than fatty acids (R·/RH)-0,6V it is catalytic .

The speed constant of the propagation reaction is in the order of 10 mol. L.s. This explains that when the oxygen content is not limiting, the vast majority of radicals are in peroxyl form.

There are several types of oxidation that implement very different action mechanisms, resulting in oxidative or hydrolytic rancidity.

The alterations leading to oxidative rancidity are self-oxidation, photo-oxidation and enzyme oxidation catalyzed by lepoxygenase. The primary products of these oxidat is are

hydroperoxides that can generate, after their degradation, compounds, low molecular weight (carboxyles, alcohols, acids) some of which are very olfactory. This stage of rancid develops in altered oil, which reduce sits market quality and directly conditions its food preservation last.

During the propagation phase, a single free radical of fatty acid can initiate the formation of many molecules of hydroperoxide. The amounts of hydroperoxides generated corresponds to the amount of hydrogen consumed during the oxidation of fatty acid chains. The rate at which hydroperoxides develop over time.

II.4.2.3.Ending[1]:

When the concentration of free radicals becomes large enough ,they can combine to complete the reaction:

$$R\cdot + ROO\cdot \longrightarrow ROOR$$

$$R\cdot + R\cdot \longrightarrow RR\cdot$$

$$2ROO\cdot \longrightarrow ROOR + O_2$$

The last of these reactions predominates, the partial pressure of oxygen is high.

The activation enthalpy of termination reactions is low, but the limit comes from the probability factor of encounter of the radicals between them.

Based on these reactions, the self-oxidation of fresh fat evolves into three distinct periods :

- The period of induction where there is stable hydroperoxide formation, the taste of fat is not altered.
- The period of active oxidation when the formation of hydroperoxides accelerates.
- The period of acceleration of secondary reactions.
- Oxygen absorption is rapid as there is an increase in the peroxide index. The taste of fat is greatly altered.

II .4.3.The impact of olive oil oxidation [2]:

II.4.3.1.Nutritional and organoleptic impact:

Degradation of fat-soluble vitamins and essential fatty acids, development of natural flavours, change of color.

II.4.3.2.Secondary impact:

Secondary oxidation products show cytoseic and mutagen effects (cases of malondialdehyde that reacts with DNA) or carcinogenic, mutagen and atherogenic effects (cases of cyclic and oxysterols).

II.4.4.Factors influencing oxidation [3]:

II.4.4.1.Physically-chemical factors:

II.4.4.1.1.Water activity (AW):

AW and the physical state of the water strongly influence the oxidative stability of a food, the maximum lipid stability observed for AW between 0.2 and 0.4.

The relative rate of lipid oxidation increases very significantly on either side of this window, on AW ranges between 0.2 and 0 or between 0.4 and 0.7. Beyond an AW of 0.7, the rate of lipid oxidation is slowed down

The influence of the AW is complex because it involves several mechanisms. Water can increase the rate of lipid oxidation by increasing the mobility of the reactants. It can also slow it down by delaying the decomposition of hydroperoxides and diluting oxidation catalysts.

II.4.4.1.2. PH:

pH affects the lipid oxidation mechanism, mainly by altering the solubility of the activity of catalysts and oxidation inhibitors.

The activity of antioxidants is usually coupled with their solubility but not only.

Thus, polyphenols are more active at basic pH or their solubility is the best. Indeed, the energy weakening of hydroxyle functions with the increase in pH facilitates the transfer of the hydrogen atom to lipid radicals.

The accessibility of fat to catalysts seen to oxidation inhibitors also modulates the oxidation rate of lipids.

II.4.4.1.2.1.Temperature:

The effect of temperature on lipid oxidation is complex and depends on the concentration of oxygen in the medium. When this 'is not limiting the rate of oxidation of lipids is generally governed by the law of Arrhenius and increases with temperature.

However, the relative contribution of the various mechanisms of initiation of lipid oxidation increases with the decrease in temperature due to the increased solubility of oxygen in the watery sentence.

Also the drop in temperature, when crystallizing the lipid function at the highest point of fusion excludes oxygen from the crystallized areas. Oxygen is then concentrated with the most established lipid fraction, which facilitates the spread of lipid oxidation. An increase in life expectancy, the antioxidant role of these compounds could more specifically protect lipoproteins from oxidative processes but their activity varies depending on their structure.

Aromas: these compounds are responsible for the flavour of the oil, overall they represent 250 to 300 ppm. The profile of aromatic oil compounds depends on its Quality... thus a fresh olive oil of good quality a profile of compounds mainly from normal bio-synthesis pathways.

*On the other hand, a lower quality olive oil would have a more complex profile that also contains volatile compounds responsible for defective taste. The main defects of olive oil are assessed by mouldy, moist, rancid and metallic aromas.

* The main reason for the appearance of undesirable taste is the formation of volatile compounds produced by over ripening of the fruit, oxidation of fatty acids introduced following a bacterial attack or storage of olives before the extraction of oils.

II.4.5. Olive oil oxidation inhibitors [3]:

Some molecules naturally present in the plant kingdom (plants) act, antioxidants knew the oils either by limiting the spread of free radicals or by controlling the activity of oxidation catalysts. Thus they delay by slowing down the discoloration or appearance of unwanted flavor due to the oxidation of the oils.

II.4.6.Anti-radical action of antioxidants [3]:

Some antioxidant-owned compounds are free radical traps, which are involved in transferring a hydrogen atom to the radical species. Antioxidants react preferentially with peroxide radicals because they are the majority radicals when olive oils expire.

Hydrogen atom exchanges significantly delay the spread as soon as oxidation is made.

The effectiveness of an antioxidant results from the presence on molecules of a low-energy bond involving a hydrogen atom. The lower the energy of this bond, the more energetically the transfer of the hydrogen atom to a radical lipid and gives its products quickly.

The effectiveness of an antioxidant also depends on its ability to reduce the energy of its radical structure so that it is not in turn a catalyst for oxidation. (So the antioxidants) (Radical antis) as well as radical antioxidants (AH°) are often stabilized by relocating electrons by resonance. They can transmit a second

hydrogen to another lipid radical and adopt a more stable non-radical molecular structure (A) or react to each other to form a stable dimer (HA-AH):

$$ROO^{\cdot} + AH \longrightarrow ROOH + A$$

$$AH^{\cdot} + AH^{\cdot} \longrightarrow HA - AH$$

The most common chemical structures involved in trapping free radicals are the hydroxide groups of phenolic derivatives, as they rapidly transfer one or two hydrogen atoms and

sleep a stable phenolic derivative, radical or not, ortho-and-para drifts are the most effective, because they give relatively stable free radicals due to the relocation of the election between forms of resonance.

The free antioxidant radical is all the more stable as the substitute grouping is larger, the antioxidant action decreasing all the time in this case:

The kinetics of the disappearance of antioxidants is directly related to the introduction of fat. Fats strongly introduced and therefore quick to form radical structures quickly consume antioxidants as long as the antioxidant is present in the system.

II.4.6.1.Control and prevention of olive oil oxidation [3]:

The oxidation of oils is dependent on the composition, concentration and activity of reaction substances, the balance of antioxidant pro-oxidant agents.

It is responsible for a loss of nutrition al quality (decrease in the content of polyunsaturated fatty acids or antioxidant vitamins) organoleptic 'formation of molecules responsible for undesirable flavors).

In addition, the reactions involved in the oxidation of olive oils involve mostly free radicals or very active forms of oxygen, which are implicated in the degenerative aging process of the body or in serious pathologies (especially certain forms of cancer, atherosclerosis, cardiovascular disease and diabetes).

Tackling the oxidation of olive oils is a considerable challenge for oil mills. To do this, it will be necessary to minimize the loss of antioxidants during the technological stages, select plants or ingredients from plants naturally rich in antioxidant molecules well incorporate antioxidants into the food action.

At the same time, the resulting oils should not be exposed to oxidation catalysts (high temperatures, or light in particular foodstuffs containing sensitizer photos).

The use of natural antioxidants belong to various chemical families (phenolic compounds, various tocopherols, ascorbic acid).

Phenolic compounds are essentially phenolic acids, flavonoids and anthocyanins. They intervene either by interrupting the propagation phase of oxidation or by activating the oxidation catalysts.

The limits to the implementation of antioxidants in food matrixes are related to their matrix to their interactions with other constituents (proteins and phenolic compounds) or to changes in color or flavor.

II.5.Chemical parameters for assessing oxidation :

II.5.1.Acid Index (I.A):

II.5.1.1.Definition:

The acid index is the number of milligrams of sodium hydroxide needed to neutralize free acids in a gram of Fat**[4].**

II.5.1.2.Principle:

Neutralizing free acids with a titled sodium hydroxide alcoholic solution.

II.5.2.Peroxide index:

II.5.2.1.Definition:

The peroxide index is a measure to estimate the amount of peroxides present in a fat. Peroxides are characteristic components of the oxidation of unsaturated fatty acids, they are determined based on their property of releasing iodine from potassium iodide in acidic environments. The released iodine is measured by the reaction with thiosulfate, knowing that 1ml of thiosulfate 0.01 N corresponds to an amount of 80 mg of oxygen attached to fatty acids **[4]** .

II.5.2.2.Principle:

It is based on the treatment of oil solution in acetic acid and chloroform by a potassium iodide solution (KI), it is the titling of iodine released by a solution titled sodium thiosulfate ($Na_2S_2O_3$).

II.5.3.Iodine index:

II.5.3.1.Definition:

This index measures overall the degree of fat insaturation by determining the number of grams of iodine attached to the double bonds present in 100g of

Lipids. Highly saturated animal fats, have iodine indices of around 45, in vegetable oils, this value reaches 150 **[4]**.

II.5.3.2.Principle:

Addition, to a test take of an iodine monchloride solution in a mixture formed of acetic acid and carbon tetrachloride.

After a given reaction time, reduce the excess iodine monochloride by adding a potassium and water iodide solution and titling the iodine released by a titled sodium thiosulfate solution.

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[2] M.Rahmani « – 18 – Les technologies de laboratoires : Méthodes d'évaluation de stabilité oxydative des lipides » Janvier / Février (2007).

[3] J.C ; Henry. C « Introduction à la biochimie et à la technologie des aliments » Vol1 Tech, Paris. (1976).

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Chapter III:

Results and discussion

Part One: Materials and Methods

III.1.Material:

The material used in our study consists of : virgin olive oil, rosemary and gallic acid.

III.1.1.Virgin olive oil:

The virgin olive oil used in the realization of our work comes from a semi-modern oil factory located in Azrou village located 30km from Bourdj bouarreridj. It is an oil extracted by first hote press. The crushed olives are of «Sigoise» variety. This oil comes from olive-growing region. January2021 .

Figure.1: Olive oil

III.1.2. Rosemary:

The Rosemary is a shrub that takes its name from the Latin ros, dew and marinus, sailor. Indeed, according to legend, Romarin is a plant that can only be found in regions where dew from the sea stretches in the early hours.In other regions, it is nicknamed "the Rose of the sea" in Latin Rosa marina which gave its name to the genus **[1].**

The chemical composition of the plant as a whole depends on where it grows and harvests and when it will be harvested in the vegetative cycle (ideal when the plant has the maximum amount of gasoline) **[2].**

The dried leaves and HE (Spain type and Morocco-Tunisia type) of Rosmarinus officinal is L. are registered as vegetable drug sat the European Pharmacopeia 11th edition.

According to the European Pharmacopeia**[3]**, the who ledried leaf of Rosmarinus officinal is L. must have a minimum content of:

- 3% of total hydroxycinnamic derivatives, expressed in rosemary acid (C18H16O8; Mt 360.3) (anhydrousdrug)
- 12 mL/kg of HE (anhydrousdrug).

In order to determine the chemical composition of the leaves and flowering tops of rosemary, we carried out a survey based on several studies.

Thus we were able to calculate average values for the molecules most often cited .

Phenolacids:

Rosmarinic acid: 1.7-2.83% on average (Figure 2)

Coffee acid: (no specified value) associated with chlorogenic acid

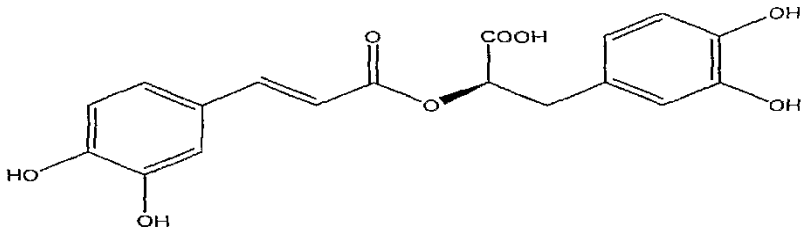

Figure 2: Rosmarinic acid **[4].**

Tricyclic phenolic diterpenes:

carnos(ol)ique acid ≈ 0.35% (Figure 3).

carnosol - picrosalvine: (variable value, up to 4.6% or majority).

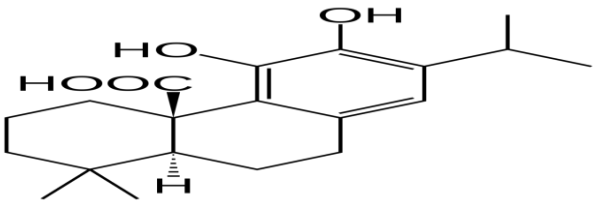

Figure 3: Carnosic acid **[5].**

Figure 4 : Rosemary.

III.1.3.Gallic acid:

Gallic acid (3.4.5-trihydroxybenzoic acid) is an aromatic organic compound ,one of the six isomers of trihydroxy benzoic acid, widely used in plants either in free form or as a component of gallotanins.It is classified as phenolicacids (or phenolicacids) because it contains both carboxylic function and phenolichydroxyls. And as it is derived from acid benzoic, it is also classified in hydroxyl benzoic acids .It is found in natural aeginames (or gallnuts), sumac ,witch hazel, tealeaves, oakbark, pomegranate skin, among other plants.

Its chemical formula is $C_6H_2(OH)_3$ (Figure 5). Salts and esters from this acid are called gallates.

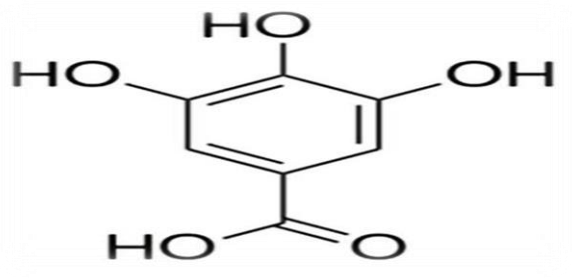

Figure 5: Chemical structure of gallic acid

Gallic acid at a concentration of 1.65 mM, accelerates the oxidation of deoxyribos isinduced by hydroxyle radicals •OH (produced byFe^{3+} - H_2O_2) **[6].**Beyond this concentration, gallic acid behaves as an antioxidant capable of reducing deoxyribosis damage caused byFe^{3+} - H_2O_2. There is also the ability to gallic acid to generate hydroxyle radicals in the presence of Cu(II) copper but in much less than tannic acid does. Conversely, antioxidant activity evidence of it stability to reduce DNA degradation. Riboflavin photosensitized is capable of degrading DNA but if gallic acid is added to it

Degradation is then limited **[7].**Tannic acid in this case completely inhibits Degradation.

Gallic acid is also a free radical trap. At a concentration of 4.17 mM, it is capable of trapping 44% of DPPH radicals and 60% of hydrogenperoxide**[6].**

III.2. Methods:

III.2.1.Acidity:

a. Principle:

Acidity is the percentage of free fatty acids in a fatty body, for example convention

It is expressed as a percentage of olec acid for olive oil (gramolec acid per 100 grams of oil). Acidity is an important criterion for appreanation olive oil to the characterization olive oil with a food characterization and is a characteristic fundamental to its commercial quality The free fatty acid content of an oil is an indicator of lipase activity, quality of fruit freshness and oil stability during storage.

$$\mathbf{RCOOH + KOH \longrightarrow RCOOK + H2O}$$

b. Procedure:

02 grams of oil are weighed in an erlenmeyer and then added : 25ml ethanol, 25mldiethylicether and a few drops of pheophthalne (colored indicator) after agitation is dose using a KOH solution (0.1) Normal the end of the dosage is marked by the appearance of a pink color that should persist for 15 seconds after agitation we note the volume of KOH.

c. Calculation formula:

$$\text{Acidity (\%)} = \frac{\mathbf{N \times V \times 282.5 \times 100}}{\mathbf{M \times 10}}$$

N : KOH normality

V : volume of KOH

M : oil mass in (mg)

282.5 : molar mass of olec acid

(a) **(b)**

Figure.6: Measure of acidity: :« a » before « b »after titling.

III.2.2.Peroxide Index:

The peroxide index of a fat body represents the number of micrograms of oxygen active preent in 1g of fat material. Active oxygen is the existing oxygen peroxide, hydroperoxide or oxide in a fatty material. It is de-mine in compliance with the AFNOR NF T60-220 standard of December 1968, the principle of which is the Following:

A test shot is set in a mix of aceic acid and chloroform ,treated with a potassium iodide solution. Iodineistitled free sodium thiosulfate solution in the presence of starch poisoning (colored indicator. In oxygen in the air, unsaturated fatty acids oxidize by giving the Peroxides. On a peroxide molecule, an oxygen molecule is attached. On both fixed oxygen atoms, only one is active and is able to oxidize the iodures.

III.2.2.1.Preparing solutions:

II1.2.2.1.1.Preparation of starch poisoning solution: it weighs about 1 g starch and dissolved in 100 ml of light water.

III.2.2.1.2.Preparation of sodium thiosulfate solution ($Na_2S_2O_3$)à 0,01N :

dissolve 2.48g of $Na_2S_2O_3$ 5 (H_2O) in one litre of distilled water Expression of results:

$$IP(\text{meqd'}O_2/\text{ Kg}) = \frac{(V - V0) \times 1000 \times C}{P}$$

V0 : Volume of sodium thiosulfate solution used for ml-white test;

V : Volume of sodium thiosulfate solution used for ml test intake;

N : Normality of sodium thiosulfate solution;

P : Mass of the gram test take.

(a) **(b)**

Figure.7: Measurement of the Peroxide index:« a » before «b» after titling.

III.3.Absorbancetoultra-violet radiation : The fat studied is dissolved in the required solvent (hexane), and then the following is determined ,the extinction of the solution at the prescribed wavelength, compared to pure solvent.

The Specific extinctions are determined from spectrophotometric readings. The law of Beer Lambert specifies that, for a given body in solution at one wavelength λ Absorption A is proportional to the thickness of the liquid layer through which it passes (the thickness "l" of the cell) by the light beam and at the concentration C of the body in the solution.

$$A(\lambda) = \varepsilon_{\lambda} . l . C$$

With :

A (λ) : absorbance at the wavelength λ.

C : the concentration, in grams per 100 ml, of the sample.

l: length of the vat.

ε_{λ}: molar extinction coefficient.

The absorbances of the olive oils ample are measure datwavelengths 232 nm and 270 nm.

Perkin Elmer LAMDA 25 The study of absorptions requires the use of an apparatus called as pectrometer: we work with two quartz cuvettes the first carries the reference (hexane) and the second carries the sample (oil dissolved in the hexane).

The spectrometer used is:

Figure 8 : UV/VIS spectrophotometre

III.4.Preparation of olive oil samples :

In order to study the influence of phenolic compounds from gallic acid and the rosemary, on the oxidative stability of virgin olive oil, two series of samples A and B grades of the same virgin olive oil were used in this study .The each included three 50ml samples of oil .These samples of virgin olive oil are distributed as follows:

- **Series A :**containing three samples of different masses of rosemary.
- **Series B :**containing three samples of different masses of gallic acid.
- **Control sample (T_1) :** virgin olive oil with out any additives exposed to the sun.
- **Control sample (T_2) :** virgin olive oil with out any additives.

Part two: Discussion of the results

III.1.The chemical and organoleptic parameters.

A good oil has a low acidity level which helps to give it greater stability against oxidation by air. This is all the more important if the oil has a high unsaturated fatty acid content. Our oil belongs to the category of olive oils called "extra virgin" in accordance with the standard set by the (C.O.I) **[8]**.≤0.8%. The factors responsible for high acidity are linked to non-compliance with the correct olive oil harvesting and manufacturing practices.

The peroxide number is used to assess the oxidation state of the oil. The alteration chemical in oils is caused by the oxidation of the air which results in the formation of peroxides.

This index could be used to control the quality of oils; because it depends on problems that can occue after harvest (modality of transport and preservation of fruit before crushing and during processing) **[9]**.

The phenomenon of fatty acid oxidation leads to changes in properties organoleptic, chemical and nutritional. These alterations affect the quality merchant of the product **[10]**.

It should be noted that the I.P. increases with the maturity of the olives, and especially following a shock thermal, as a result of freezing or a faulty manufacturing process. The improper or prolonged storage is also one of the causes of this increase setting.

It is a parameter that determines the degree of unsaturation of fatty acids entering the composition of fat, it varies with the wavelength of the incident light as well as with temperature.

The pH is involved in the mechanism of lipid oxidation. Oils that exhibit a pH less than 4 are acidic oils. Storage in open air changes the pH. This parameter allows the total elimination of water and volatile products.

The results show that all samples have a water content less than standards established by the Official Journal and the International Olive Council. Humidity, causes an increase in the growth of yeasts and molds during storage.

Regarding the absorbance in ultraviolet (A), it can provide indications on the quality of a fat, on its state of modification due to the processes technological **[11].**

The results show that all samples have an absorbance value in the ultra purple below the standards set by the International Olive Council. So these oils do not contain secondary products such as linoleic hydroxyperoxide ,unsaturated ketones and diketones. The results

would be linked to several factors such as late harvest of olives, excessive exposure of olives and the extracted oil to oxygen in the air and in light, also to heating of the dough during crushing.

From these results gathered in Tables 1 and 2 and according to the C.O.I standard, we classifies our oil as extra virgin oil.

The results obtained allow us to say again that:

- The olive oil studied is not very acidic and is suitable for consumption,
- The peroxide number agrees with that quoted in the standards; allowing to qualify it as good,
- The refractive index does not comply with C.O.I. ; so she is not totally pure,
- The water content is within the standards,
- Absorbances in ultraviolet met standards,
- The organoleptic characteristics show that the olive oil studied does not presents no anomalies.

Table 1: Summary table of results for different chemical and physical characteristics.

Settings	Calculated value	Standard: C.O.I
Acidity (%)	0.480%	< 0.8 (extra virgin)
Peroxide index (PI) (meq of O2 / Kg of oil).	17.5	< 20
Réfractive index	1.474	1.4669 – 1.4679
Water content (H%)	20%	< 0.2
PH	3.61	/
A232	0.730	<2.24
A270	0.210	< 0.30

Table 2: Results of the organoleptic characteristics.

Aspect	Clear
Color	yellow
Odour	Strong
Flavor	Very good
Taste	Good

III.2. Monitoring of the oxidation of olive oil by chemical methods

III.2.1.Acidity

Table 3 and figure give the distribution of the acidity values of the samples of virgin olive oil according to the mass of the materials added according to the duration of storage.

Table 3. Evolution of acidity over time

Sample	**Mass (g)**	**Storage time**						
Witness 1	**/**	**0 days**	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
		0.480	0.570	0.649	0.800	0.889	0.946	0.98875
Witness 2	**/**	0.480	0.530	0.560	0.610	0.640	0.678	0.678
Serial A (Rosemary)	**1 g**	0.480	0.282	0.282	0.285	0.282	0.310	0.339
	2 g	0.480	0. 268	0.240	0.240	0.249	0.282	0.2825
	4g	0.480	0.226	0.218	0.220	0.224	0.240	0.31075
Serial B (gallic Acid)	**0.05 g**	0.480	0.290	0.339	0.341	0.347	0.324	0.339
	0.1g	0.480	0.339	0.380	0.385	0.390	0.353	0.36725
	0.5g	0.480	0.395	0.423	0.450	0.470	0.522	0.5226

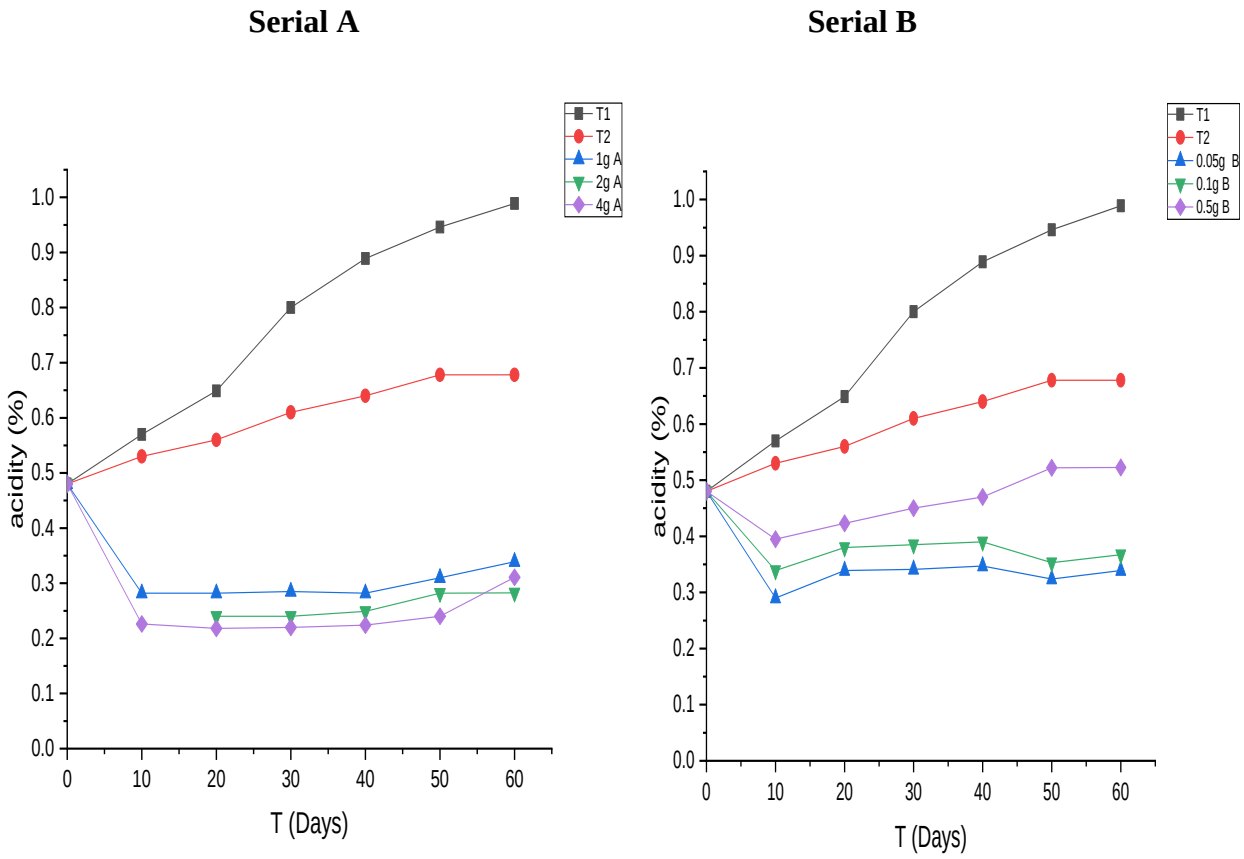

Figure.09. Evolution of acidity over time: Serial A: rosemary, Serial B: gallic Acid.

We note that the initial acidity of our oil which is 0.480% complies with the limits established by the C.O.I **[12]**, which are between 1 and 3.3%.. If the acidity exceeds these limits is because it is due to the late harvest of the olives and their storage for a long duration because of the reception rate which is lower than the trituration, the degradation will be all the more accentuated as the storage will be long (more than48 hours) and performed in poor conditions. This causes heating of the olives and starts the fermentation process by increasing the acidity level. The free fatty acids result from the action of lipases on triglycerides, or any other hydrolytic activity of these triglycerides which may occur before, during or after the crushing olives **[13]**. However, a high level of acidity can also be attributed to the advanced state of ripeness of the fruit, Dugo et al **[14]** showed that the harvest early produces oils with an acidity of less than 1%.

According to D. Boscou **[15],** factors adversely affecting the quality of an oil olive oil may be present even in the early stages, for example, during formation of oil in the fruit.

Abnormalities during the process of biosynthes is, microbial activities and environmental conditions are all related to the formation of the oil at high acidity.

From the results reported in Table 3 and reported in Figure 10, we note that all the acidity values of olive oil samples to which is added gallic acid seem to have the highest values because of the acid function of the antioxidant used for these samples. This variation in acidity(increase) is slight for all series of oil samples used.

In any case, the acidity of the oil studied does not vary significantly with the addition of antioxidants. These results are in agreement with those obtained by N. Denisse **[16]**who found that the acidity of sunflower, nut and soybean oils to which are added 80 ppm and 160 ppm of the phenolic extract of vegetable water remains constant for a storage time of 22 days at 60 ° C. This tendency was noticed by S.Fodil **[17]** during of the study of the effect of βcarotene and vitamin E on the oxidative stability of three types of virgin olive oil.

III.2.2 Peroxide index

Table 4 and Figure 10 give the distribution of the values of the peroxide number of samples of virgin olive oil according to the mass of the materials added according to the storage time.

Table 4. Evolution of the peroxide index over time.

Sample	**Mass (g)**	**Storage time**						
Witness 1		**0 days**	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60 days**
		17.5	26	30	34	36	45.5	57.5
Witness 2		17.5	19.5	23	25	26	27.5	32
Serial A (Rosemary)	**1 g**	17.5	10.5	7.5	8	8.1	4.5	4.5
	2g	17.5	9.5	7	9.1	9	3.5	4
	4g	17.5	8	6.5	6.8	7	2.5	3.5
Serial B (gallic Acid)	**0.05 g**	17.5	15	10.5	13	13	11	11
	0.1g	17.5	5.5	2.5	2.6	2.8	4	5
	0.5g	17.5	1	3.5	3.5	3.5	4.5	4.5

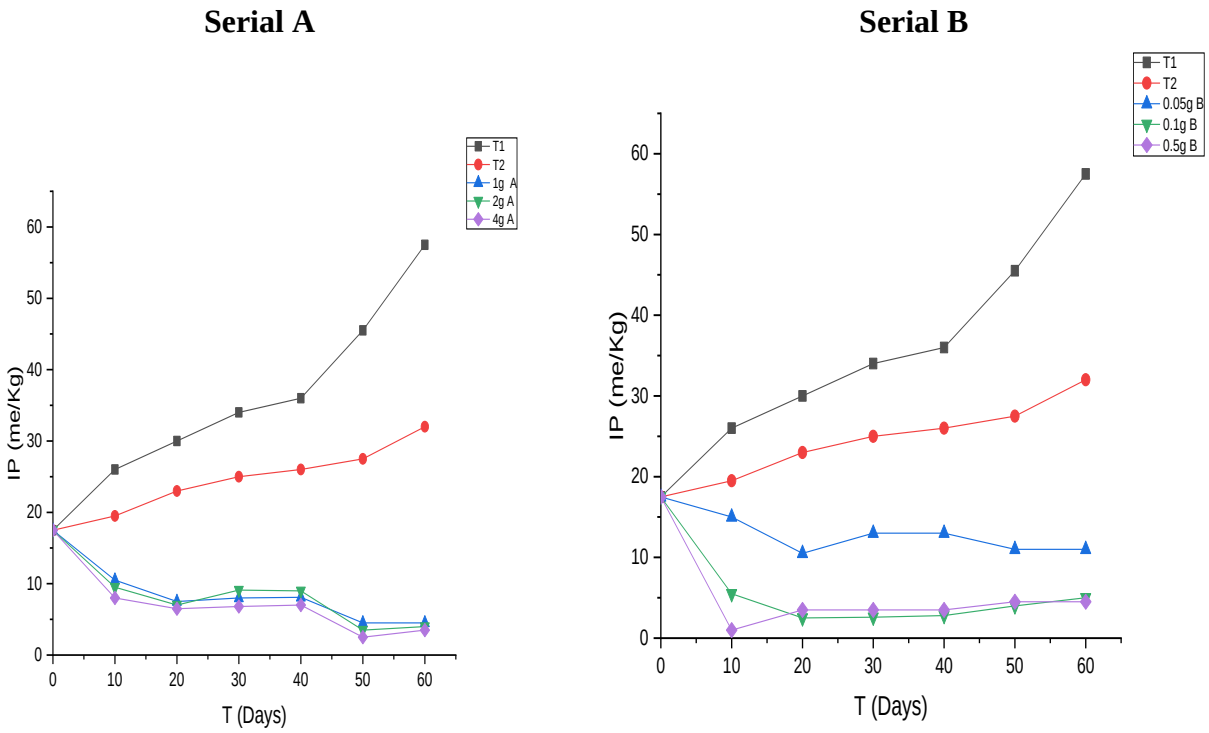

Figure.10. Evolution of the peroxide index over time: Serial A: rosemary,

Serial B = gallic acid.

Oxidation of olive oil begins after the olives are picked from the tree, and continues during fruit storage and processing. The first products formed by the attack of oxygen, activated on the double bonds of fatty acid chains, are unstable peroxide compounds, and hydroperoxides whose structure will depend on the nature of the fatty acids attacked (mono-, di-, tri- or polyunsaturated acids).

The determination of the peroxide number is the most suitable method for measuring of these peroxide compounds.

Analysis of the results of Table 4 shown in Figure 11 shows an evolution of the peroxide value of all the series of samples as well as that of the two controls in depending on the storage time.

The initial value of the peroxide number of the control oil sample is 17.5 meq / kg.

After 60 days of storage, it reaches a value of 57.5 meq / kg. The other samples of oil to which are added the antioxidants used during this work as well as that the witness shielded from light seem better protected than the witness exposed to the sun against oxidation. The values of the peroxide index obtained after a period of storage for 60 days vary between

17.5 meq / kg and 11 meq / kg respectively for samples to which rosemary is added. They correspond to values optimal for protecting olive oil from oxidation. The results obtained with the phenolic compounds of rosemary are in accordance with the results obtained by L. Machlin **[18]** as well as those of M. Baldioli et al **[19]** who found that the stability of oils is well correlated with the content of total phenols and the presence of a high level natural antioxidants, the most important of which are tocopherols. While the results found with the incorporation of gallic acid are optimum with the sample(0.5 g), this is in agreement with the results of F. Pirisi et al **[20]** who showed that the oxidation stability, evaluated by the Swift test with the Rancimat, would not be correlated with the content of phenolic compounds. The same goes for Cillard et al **[21]** who find that the increasing concentrations of antioxidant added in the medium could be responsible for a prooxidant effect, as has been shown for α-tocopherol. In effect, if the concentration of the radical form of the antioxidant produced by oxidation increases a lot, it can behave as an initiator of lipid peroxidation

according to the reaction proposed by H. Chimi et al **[22]:**

ROO ° + ArOH ROOH + ArO ° (ArOH: Antioxidant)

ArO ° + RH ArOH + R °

From the twentieth day of storage, the peroxide value of the oil samples olive grows over time. This increase is much greater than that obtained during our study.

Beyond this period, these phenolic compounds seem to exert a better antioxidant activity. These results are in agreement with those obtained by I. BenTekaya and M. Hassouna **[23]** who found a prooxidant effect of chlorophylls in light but antioxidant in the dark, in the presence of other compounds such as β-carotene.

This better antioxidant activity may also be due to the composition of the seed olive rich in oleuropein, the latter is also the main phenolic compound and mostly olive leaves **[24]**, as well as its chemical structure with two phenol functions in ortho position. These results are consistent with those obtained by H.

Chimi et al **[25]** who have shown that if the antioxidant used is a sterically phenolhindered or a para or ortho-diphenol, the ArO ° radical derived from the antioxidant does not participate not to the propagation of the chain reaction, these radicals then recombine very quickly between them to give quinones and the starting ortho-diphenol (products not radicals).

We note that the olive oil samples incorporating the antioxidant (gallic acid) and rosemary are the least peroxidized, with a value of the peroxide value at the end of the shelf life (60 days) equal to 3.5 meq / kg and respectively for the masses 0.05g of gallic acid and 0.05 g for rosemary. These results can be explained by the synergistic effect and by the structure of gallic acid which has three phenol functions which can also yield three hydrogens to the radicals peroxyls (RO °) and hydroperoxyl radicals (ROO °). Therefore, it is likely to stabilize three radical functions.

In conclusion, we can say that the peroxide number represents one of the parameters quality of olive oil, but cannot be an indicator of the oxidative stability of the oil.

These results agree with those of Kiritsakis et al **[26]** who studied three samples different olive oils and found that the oil that had an initial value of the lowest peroxide was less stable than the others.

III.3. Monitoring of the oxidation of olive oil by physical method

III.3.1 Absorbance to UV radiation

All fatty substances contain epoxides and hydroperoxides in amounts greater than or less important.

Isomerization reactions result in the formation of dienes and trienes conjugates that absorb light between 225 and 280nm **[27].** Indeed, conjugated dienes and the primary products of fatty acid oxidation are formed by rearrangement of double bonds of the alkyl radical of polyunsaturated fatty acids when they have a structure Conjugated diene absorb light in the vicinity of 232 nm. The conjugated trienes (in the case of the presence of fatty acids with three conjugated double bonds) and the products secondary oxidation, such as α-unsaturated aldehydes and ketones, absorb light around 270 nm. The determination of the absorbance in the vicinity of these two values makes it possible to detect and evaluate the quantities of oxidation products: the higher the extinction at 232 nm is the stronger it is peroxidized. Likewise, the greater the extinction at 270 nm, the more it is rich in secondary oxidation products **[28]**.

Table 5 and figure 11 give the distribution of the absorbance values at 232 nm samples of virgin olive oil according to the mass of the materials added according to storage time.

Table 5. Evolution of absorbance at 232 nm.

Sample	Mass (g)	Storage time						
Witness 1	/	**0 days**	**10 days**	**20days**	**30 days**	**40 days**	**50 days**	**60 days**
		0.730	0.780	0.785	0.770	0.775	0.778	0.779
Witness 2	/	0.730	0.730	0.730	0.790	0.236	0.242	0.238
Serial A (Rosemary)	**1 g**	0.730	0.710	0.649	0.660	0.520	0.531	0.523
	2g	0.730	0.672	0.659	0.663	0.542	0.557	0.543
	4g	0.730	0.763	0.797	0.745	0.650	0.649	0.651
Serial B (Acid gallic)	**0.05 g**	0.730	0.788	0.800	0.798	0.395	0.765	0.400
	0.1 g	0.730	0.732	0.731	0.751	0.226	0.224	0.229
	0.5 g	0.730	0.673	0.637	0.650	0.348	0.371	0.349

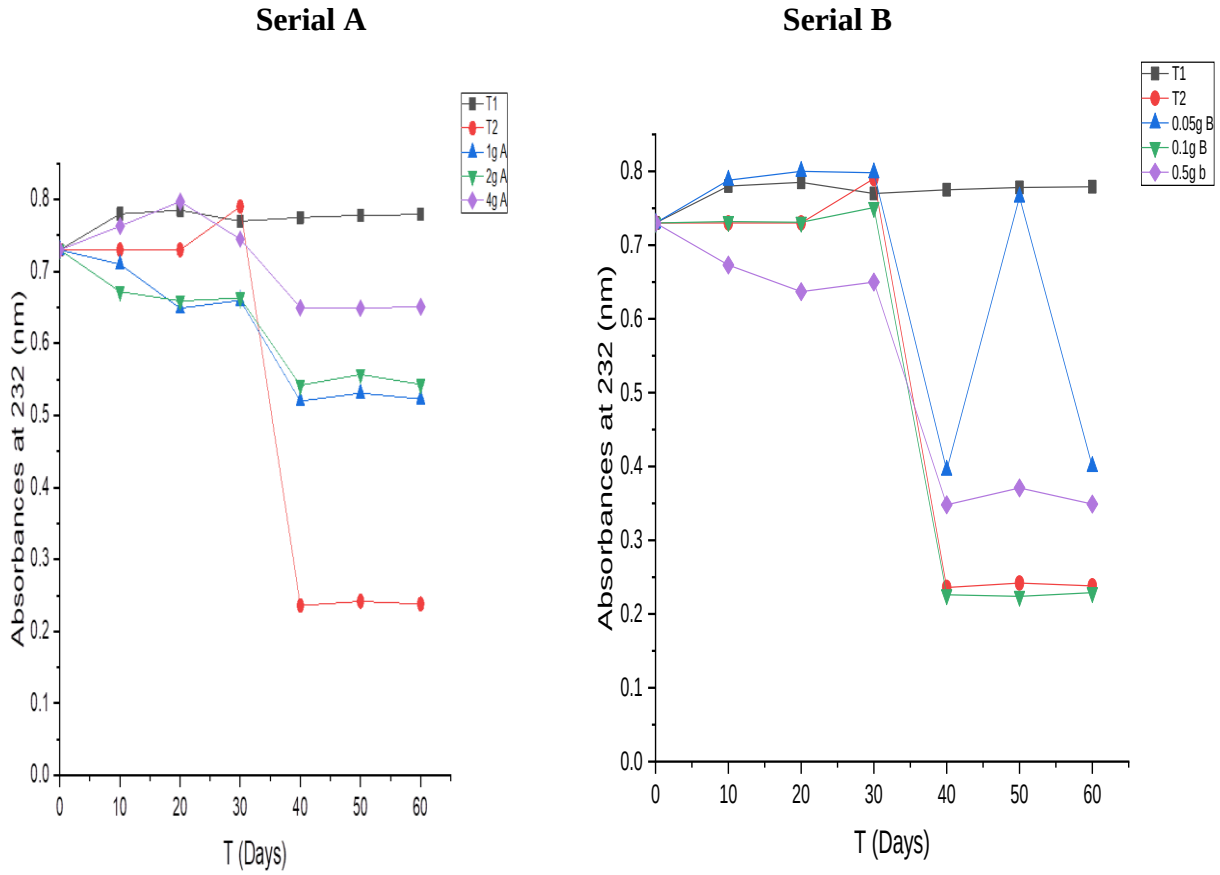

Figure .11. Evolution of absorbance at 232nm over time: Serial A: rosemary, Serial B = gallic acid.

From Table 6 and Figure 12, absorbance at 232 nm tend to increase. Smaller values are seen in the olive oil series containing the acid gallic. In addition, all the values of the samples of the olive oil series containing rosemary are smaller than those of control 2 which show a slight increase throughout the storage period. They are therefore at the first stage of propagation which corresponds to the formation of peroxides but not hydroperoxides.Oxidation of lipids increases with the concentration of compounds resulting from degradation hydroperoxides, this is confirmed by an increase in the absorbance values at270 nm. The extinction at 270 nm is used to determine the proliferation of oxidation, the side products of oxidation and in particular α-diketones. This evolution of the absorbance for the olive oil samples is given in Table 6 and shown in figure 13.

Table . 6. Evolution of absorbance at 270 nm.

Sample	**Mass (g)**	**Storage time**						
Witness 1	**/**	**0 days**	**10 days**	**20 days**	**30 days**	**40 days**	**50 days**	**60days**
		0.210	0.244	0.248	0.321	0.370	0.410	0.372
Witness 2	**/**	0.210	0.210	0.210	0.250	0.284	0.354	0.282
Serial A (Rosemary)	**1g**	0.210	0.222	0.231	0.230	0.275	0.365	0.280
	2g	0.210	0.210	0.213	0.200	0.221	0.250	0.253
	4g	0.210	0.230	0.234	0.236	0.242	0.328	0.244
Serial B (gallic Acid)	**0.005g**	0.210	0.208	0.202	0.202	0.263	0.353	0.272
	0.1g	0.210	0.219	0.232	0.230	0.281	0.339	0.284
	0.5g	0.210	0.278	0.304	0.300	0.297	0.313	0.301

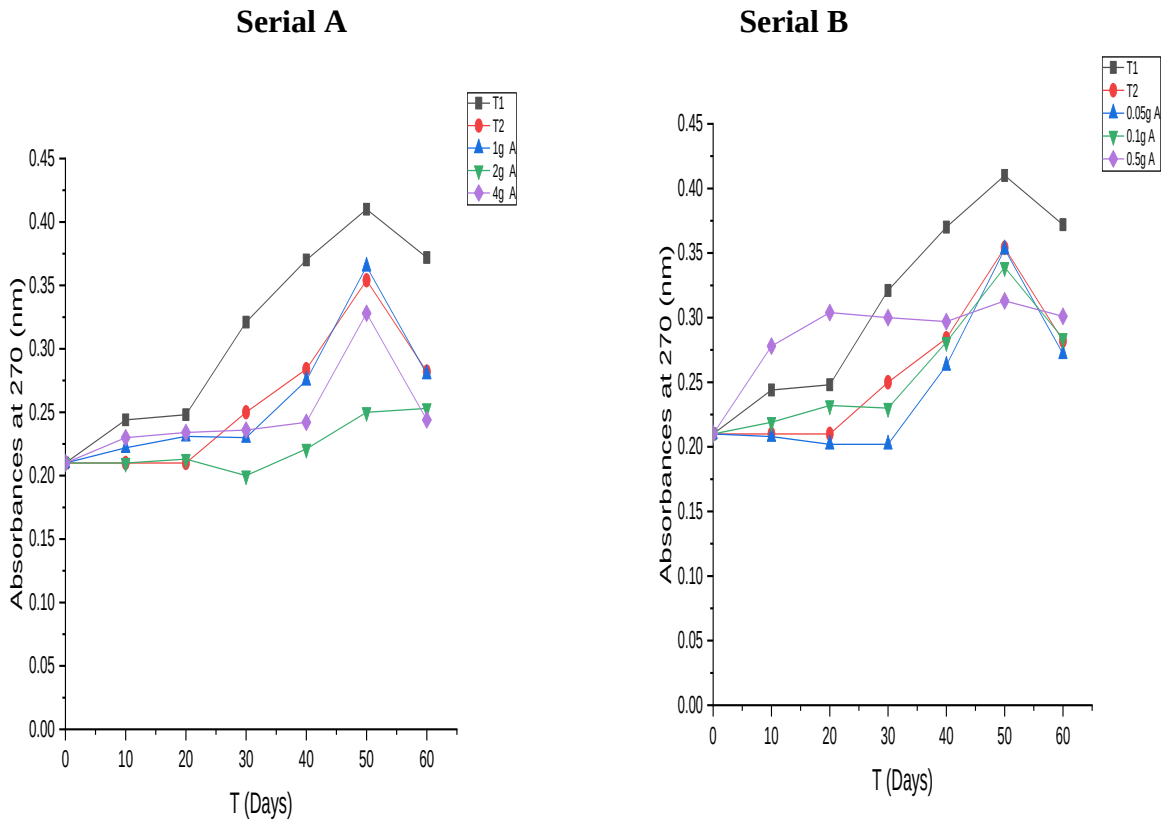

Figure .12. Evolution of absorbance at 270 nm over time: Serial A Serial A: rosemary, Series B = gallic acid.

We notice a slight increase for all samples after 60 days, this could mean that the spread of olive oil oxidation has not reached its in al stage (decomposition of hydro peroxides). This resistance to oxidation can be due to storage conditions (darkness) because the mechanism of photo-oxidation is much faster than that of auto-oxidation. We found that after two months of storage, secondary oxidation compounds are predominant in stored oils under diffused light, while in oils stored in the dark, these are the predominant primary oxidation compounds **[29]**.

The results found for this analysis coincide with the values of the peroxide number which tend to increase slightly during the entire storage period, this could mean that the spread of olive oil oxidation has not reached its final stage (decomposition of hydro peroxides). This resistance to oxidation can be due to the storage conditions (darkness) because the photo-oxidation mechanism is much faster than that of auto-oxidation. Caponio et al [30] found that after two months of storage, secondary oxidation compounds predominate in oils stored under diffused light, as evidenced by A270, while in oils of olive stored in the dark, it is the primary oxidation compounds that predominate, as evidenced by the K232.

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General conclusion.

Olive oil is the main source of fat in diets Mediterranean foods. This type of diet has often been associated with better resistance to certain diseases, including cardiovascular diseases and diseases degenerative. Many scientific studies have therefore focused on the olive oil to understand the mechanisms of action that can be explain these phenomena. The first explanation is its specific composition in fatty acids . the proportion of saturated fatty acids is very low (14%) while fatty acid the majority is oleic acid, which is a mono-unsaturated fatty acid. oleic acid gives the oil a certain stability because it is not very sensitive to oxidation. Essential polyunsaturated fatty acids are also present in interesting proportions in oil and their benefits, especially at the protection against cardiovascular diseases and cancers, have largely been studied over the past few decades.

On the other hand these molecules are very sensitive to oxidation which could cause a premature rancidity of oils. To protect its main molecules fromoxidation, the olive has developed means of defense: phenolic compounds. These compounds are partially found in oil (although most compounds phenolics is very water soluble and is therefore eliminated in margins) which allows extend its life. They protect the oil from oxidation, it's mostly on their in vivo role that scientists have worked on. Indeed, the radicals generated by the oxidizing stress have often been identified as the cause of major causes of death in developed countries: cardiovascular disease and cancer.

Their stabilization therefore seems to be a major issue and this explains the enthusiasm increasingly important for antioxidants. Incorporation of plants such as the rosemary in extra virgin olive oil has proven their ability to stop oxidation of this oil while keeping its acidity as it is with the formation braking secondary compounds such as hydroperoxides . this study was done by comparing the effect antioxidant of the plant in question by comparing it with the effect of a Synthetic antioxidant known as gallic acid.

At the end of this study it is recommended to use natural antioxidants which have shown their effectiveness as retardants of olive oil alteration for replace synthetic antioxidants that often present toxicities. Indeed, this method can be recommended for olive growers as it ensures the slowing down of propagation chain radical reactions that occur in oil during storage.

Summary

In our work, two antioxidants, one of which are plants, name rosemary, and the other is a synthetic compound which is the gallic acid known from the literature for its very high antioxidant effect. Mass quantityes added to the extra virgin oil were placed under storage conditions in the dark for 60 days. The results obtained with respect to acidity, peroxide index and specific extinction showed the very effective effect of the materials Incorporatedin question to stop the deterioration of extra virgin olive oil in the formation of secondaryoxidation compounds.

Keywords: olive oil, galic, rosemary, antioxidants.

Résumé

Dans notre travail on a fait appel à deux antioxydants dont l'un est une plante ,à savoir leromarin et l'autre est un composé synthétique qui est l'acide gallique connu dans lalittérature par son effet antioxydant très élevé .Des quantités en masse sont additionnéesdans l'huile d'olive extra vierge sont mises sous des conditions de stockage dansl'obscurité pendants 60 jrs . Les résultats obtenus en ce qui concerne l'acidité, l'indice deperoxyde et l'extinction spécifique ont montré l'effet très efficace des matériauxincorporés en question de stopper la détérioration de l'huile d'olive extra vierge en matièrede formation des composés secondaires d'oxydation.

Mots clés : huile d'olive, ail, romarin, antioxydants.