



# Determination of kinetic parameters using Rancimat analysis

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### ***Abstract***

The physicochemical quality parameters, the total phenols contents and the oxidative stabilities at 120 to 160 °C of commercial extra-virgin olive oils were assessed, allowing to confirm the quality grade as well as to group them according to the total phenols contents as low ( $88\pm 7$  mg CAE/kg), medium ( $112\pm 6$  mg CAE/kg) and high ( $144\pm 4$  mg CAE/kg) phenols levels. The results pointed out that oils with higher total phenols contents were more thermally stable. Kinetic and thermodynamic parameters were determined using the activated complex/transition-state theory, showing that their values did not significantly differ for the two highest total phenols contents, suggesting a hypothetically threshold saturation of the beneficial effect. High total phenols content would imply a significant more negative temperature coefficient, higher temperature acceleration factor, greater activation energy and frequency factor, higher positive enthalpy of activation, lower negative entropy of activation and greater positive Gibbs free energy of activation. The results confirmed that the lipid oxidation was a non-spontaneous, endothermic and endergonic process with activated formed complexes structurally more ordered than the reactants. A negative deviation from the Arrhenius behavior was observed for all oils being the super-Arrhenius behavior more marked for oils with lower total phenols contents.

**Keywords:** Rancimat test; Oils thermal stability; Energy of activation; Enthalpy of activation, Entropy of activation; Free Gibbs energy

## ***Resumo***

Neste estudo foram avaliados os parâmetros de qualidade físico-químicos, os teores de fenóis totais e as estabilidades oxidativas na gama de 120 a 160 ° C, de azeites comerciais virgem extra, permitindo confirmar a classificação de virgem extra, bem como agrupá-los de acordo com teores totais de fenóis em baixo teor ( $88 \pm 7$  mg CAE/kg), médio teor ( $112 \pm 6$  mg CAE/kg) e elevado teor ( $144 \pm 4$  mg CAE/kg) de fenóis. Os resultados demonstraram que os azeites com maior teor de fenóis totais foram mais estáveis termicamente. Parâmetros cinéticos e termodinâmicos foram determinados com base na teoria do complexo/estado de transição ativado, mostrando que seus valores não diferiram significativamente para os azeites com maiores teores de fenóis totais. Azeites com um alto teor de fenóis totais possuíam um coeficiente de temperatura significativamente mais negativo, maior fator de aceleração de temperatura, maior energia de ativação e fator de frequência, maior entalpia positiva de ativação, menor entropia negativa de ativação e maior energia livre de Gibbs. Os resultados confirmaram que a oxidação lipídica é um processo não-espontâneo, endotérmico e endérgico em que os complexos formados são estruturalmente mais ordenados do que os reagentes que lhes deram origem. Um desvio negativo ao comportamento de Arrhenius foi observado para todos os azeites estudados sendo o comportamento supra-Arrhenius mais notório para azeites com menor teor de fenóis totais.

**Palavras chave:** método Rancimat; estabilidade térmica de azeites; energia de ativação; entalpia de ativação, entropia de ativação; energia livre de Gibbs

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## List of abbreviations

A: frequency factor

A<sup>•</sup>: radical of anti-oxidant

AH: anti-oxidant

BHA: butylated hydroxyanisole

BHT: butylated hydroxytoluene

E<sub>a</sub>: Activation energy

EVOO: extra-virgin olive oil

FA: free acidity

IOC: international olive council

IP: induction period

OSI: oxidative stability index

PV: peroxide value

SD: standard deviation

TP: total phenol

VOO: virgin olive oil

$\Delta H^{++}$ : enthalpy

$\Delta S^{++}$ : entropy

## ***1. Framework***

Olive oil is a highly appreciated food, a necessity for some people being the key element of the Mediterranean diet and culture, which possesses several nutritional and healthy recognized benefits (*Buckland, 2015*). The olive and olive oil have a significant agro-economic impact in several worldwide in many regions. According to the United Nations Food and Agriculture Organization (*FAO*), Italy, Spain, Greece and Portugal are the principal European olive oil producing countries. These four countries produce almost all of the European olive oil and 80% of the world's production of olive oil (*Gonzalez-lamas, 2019*).

Olive oil is sensitive to oxidation such like other vegetable oils, being sensible to oxidation during storage due to photo-oxidation and auto-oxidation and the auto-oxidation depends on several factors which are, free fatty acids, the presence of metal ions and water, the unsaturation degree of the oil; the packaging used, the ambient temperature in which county, and the daylight exposure for transparent packaging and the oxygen in the atmosphere some factors accelerate the oxidation but there are not necessary and sufficient elements to trigger the oxidative phenomenon and due to its healthy image physicochemical tests should be done when needed .

The usual olive oil present high contents in antioxidants (like vitamin E, polyphenols, etc.), which contributes to minimize the free formation of radical, and the oxidative stress that have been related to different chronic diseases so the main aim of this investigation will be measuring the resistance of different olive oils to oxidation under accelerated conditions aging conditions (e.g., high temperature and air flow rates). For this, the Rancimat methodology will apply aiming a deeper knowledge of the oxidation process, kinetic thermodynamic parameters like the enthalpy and entropy will be assessed based on the Rancimat data. Which allow the evaluation of different properties of the olive oils as well as to infer about the nature of the oxidation process.

## 1.2. Introduction

A thorough knowledge of the ability to oxidation of vegetable oils has become a topic of great importance, since these oils may be used in food, pharmaceutical, and industrial applications (paints, rubber, base lubricants, cosmetics, etc.). One of the most critical factors affecting the shelf life of oil is lipid oxidation (*Barmak, 2011*). It has a direct effect on consumer acceptance and adversely influences lipids, proteins, carbohydrates, pigments, and fat-soluble vitamins, causing development of off-flavour, loss of nutritional value, discoloration, and the production of potentially toxic compounds (*Abdullah, 2007*). Different experimental methodologies have been developed to assess the resistance of oils and fats to oxidation (*Amir, 2013*). Among the available techniques, the Rancimat test is the most commonly used, due to its simplicity and good repeatability. Moreover, this method may be seen as a green technique since it does not require the use of organic solvents. The Rancimat test is based on conductivity measurements of water which collects the volatile organic acids produced in an accelerated oxidation process (*Amir, 2013*). this test provides the determination of induction period (IP) or oil/oxidative stability index (OSI), which happens prior to the fast oil deterioration (*Farhoosh, 2008*). Kinetic modelling implies that changes can be assessed sigma thematical models containing characteristic of kinetic parameters, such as the rate constants and activation energies (*Boekel,2001*). For that, the oxidative stability of an olive oil must be evaluated at different temperatures. Traditionally, the Arrhenius equation is employed to compare reaction rates at various temperatures (*Asadauskas, 2009*). the aim of the present study was to investigate the oxidation kinetic parameters of olive oils with different contents of total phenols.

### ***1.2.1. From olive to olive oil***

Today there are nearly one billion olive trees (*Olea europaea* L.) grown throughout the world and that on almost every continent. More than 90% of the olive trees are grown in the Mediterranean basin, the main producer of olive oil is the Union European which represents more than 70% of the world production of olive oil. In the European Union, only 7 countries produce olive oil: Spain, Italy, Greece, Portugal, France, Cyprus and Slovenia. Among the major producing countries (producing more than 20 000 tons of olive oil per year), we can quote Tunisia, Turkey, Syria, Morocco, Algeria, Argentina, Jordan. The main consumers of olive oil are producing countries, there are more than one hundred varieties of olive trees, grown according to their end purpose. Olives can have two great uses: the first is the use as a whole fruit or called "Table olives", the second is for the production of olive oil. World production of table olives is about one million tonnes or 10% of the total olive harvest. The vast majority of olives are therefore used for the production of olive oil, after a sharp increase in the 1990s, world production olive oil has remained relatively stable since the beginning of the 2000s with an annual production between 2, 4 and 3.2 million tons (IOC, 2009). Variations observed from one year to the next are due to both climatic conditions and the fact that olives grow only on 2-year-old timbers (biannual harvest). The production world is greatly influenced by those of Spain and Italy which represent they alone nearly 2/3 of world production

#### ***a. Production method of olive oil***

According to the IOC (*Vossen, 2007*), olive oil is an oil obtained from the fruit of the olive tree, the exclusion of oils obtained by extraction with solvents, by re-esterification procedures, or by any mixture with other types of oils. Unlike other vegetable oils or other products such as wine, olive oil does not require any refining step and no chemical transformation. Thanks to this procedural simplicity, olive oil has been manufactured since antiquity. The technique has undergone many changes over time that can be grouped into two broad categories: developments relating to the grinding of olives and developments relating to the separation of different phases. Between these two main stages, the olive paste is kneaded in order to be homogenized and allow the coalescence of the oil droplets. When they arrive at a mill, the olives are weighed and then usually in a washer-stripper system that will clean them and allow them to remove impurities (soil, pebbles, leaves ...) before grinding. Grinding (or trituration) olives is intended to destroy the cells of the olives so that they can then release their content. At this

stage of the process the olives are reduced to a more dough or less homogeneous which will have to be kneaded. In addition to the role of homogenization of the dough, the tray kneading allows the coalescence of the oil drops: the microdroplets of oil which have just been released from their cell lipovacuoles will come together to form Larger drops that will be easier to extract from the dough. The kneaded dough goes then be pressed or centrifuged horizontally to separate the solid phases and liquids. The solid phase contains the remains of the nuclei as well as the skin and pulp of the olives devoid of its oil. This solid phase is called "pomace" and is one of the most two main co-products in the production of olive oil. The liquid phase is a mixture of water and oil that must be separated. This is done either by simple decantation gravitational, either by centrifugation. In both cases the aqueous phase also called "Margines" is separated from the oil and is the second co-product of the production of olive oil. The fatty phase is pure olive oil; like no treatment or any additional chemical reaction is needed, the olive oil is edible in the state.

### ***b. harvesting olives***

The olive harvest period goes from September to February depending on the use of olives (table olives earlier than for oil) as well as depending on the variety of olives. Conditions climatic conditions and the region of production also have an impact on the speed of maturation of olives and therefore on the optimal harvest period. There are many olive harvesting techniques that vary according to the final destination of these olives, the nature of the soil and the area of the holding. The traditional method is harvesting by hand; it's the most respectful of the tree but the harvest is tedious and very long so this technique is no longer used only for table olives (because they must not be damaged). The most Commonly used in Provence is manual comb picking. Olive growers place a net on the ground and use a comb that will pull olives from the branch and make them fall on the net. There are now mechanical comb systems equipped with an engine rotating the combs at the end of a telescopic handle. This technique allows a faster harvest of olives and remains little traumatic for the olive trees. In Spain or Italy, the most widely used technique on large farms is that by vibration of branches: metal clips clasp the trunk of the olive tree and a vibration high frequency will be applied to the trunk. The ripe olives will then fall from the tree and can be used for oil production. The main disadvantage of this system, besides its cost to purchase, is the damage that it can cause to the young branches of olive.

### ***1.2.2. Olive oil commercial grades***

Olive oil is an oil derived solely from the fruit of the olive tree (*Olea europaea L.*) by mechanical methods or by other physical means under special thermal conditions. It should not be subjected to other treatments than fruit washing, pressing, dough preparation, separation of solid and liquid phases, decantation, centrifugation and filtration.

Virgin olive oil is therefore the oily juice of the olive fruit. Well treated, it preserves intact the flavor, aromas and vitamins it contains.

This definition is; however, incomplete and other criteria divide the oils into different subcategories (*United Nations Conference on Trade and Development, (UNCTAD.), 2005*).

According to the EU regulations and IOC recommendations (*IOC, 2002*) an olive oil is classified as:

- **Extra virgin olive oils EVOO** (FA  $\leq$  0.8% oleic acid, PV  $\leq$  20 mEq O<sub>2</sub>/kg, K<sub>232</sub>  $\leq$  2.50, K<sub>270</sub>  $\leq$  0.22,  $\Delta K \leq$  0.01; fruity median intensity greater than 0 and median intensity of defects equal to 0);

- **Virgin olive oil VOO** (FA  $\leq$  2.0% oleic acid, PV  $\leq$  20 mEq O<sub>2</sub>/kg, K<sub>232</sub>  $\leq$  2.60, K<sub>270</sub>  $\leq$  0.25,  $\Delta K \leq$  0.01; fruity median intensity greater than 0 and median intensity of defects greater than 0 and lower than 3) or Lampante olive oil LOO (in the other cases).

Refined olive oil: olive oil obtained by refining virgin olive oils. Its oleic acid acidity is at maximum 0.3 grams per 100 grams and its other characteristics correspond to those provided for this category.

Olive-pomace oil: This oil is obtained by treatment with solvents or other physical processes of olive pomace, excluding oils obtained by processes of olive oil re-esterification and any mixture with oils of other kinds (*International Olive Council, 2002*).

### 1.2.3. *Benefits associated to olive oil*

In the past, olive oil was the only vegetable oil. Apart from its use in the culinary field. Olive and oil were involved in several other applications:

- Therapeutics: Cough remedy. Constipation, prevention against sunburn, whitening teeth and gum care.
- Cosmetics: Manufacture of soap, skin care and hair.

Apart from the oil, all the by-products of the olive tree were used in daily life:

- The infusion of the olive leaves was used as diuretic, astringent, hypoglycemic, hypotensive.
- Powdered olive twigs and peels were used as relaxing and tonic in hot baths.
- Olive cake, leftover from the pressure of the olives, which can be used after fuel removal or reused as fertilizer.

### 1.2.4. *Composition of olive oil*

The composition of olive oil changes according to variety, climatic conditions and geographical origin. Olive oil is composed of 98% to 99% triglycerides. It also contains free acids, the proportion of which is variable and depends on the hydrolysis of triglycerides. Olive oil is mainly composed of monounsaturated fatty acids (72%) with 14% polyunsaturated fatty acids and 14% of the saturated ones (*Harwood, 2000*).

According to (*Benrachou, 2013*) the compounds can be classified into two main groups:

- Saponifiable substances (triglycerides, fatty acids,) (from 96 to 98% of the oil);
- Unsaponifiable substances (from 2 to 4% of the oil).
- The saponifiable fraction

As in most vegetable oils, the saponifiable fraction represents 98.5% to 99% of the oil (*Roehly, 2000*), this fraction is essentially composed of:

- Triglycerides:

They are esters of fatty acids and glycerol. Triglycerides are the main component of olive oil, about 98% (*Ryan, 1998*).

- Fatty acids:

Olive oil is characterized by the predominance of mono unsaturated fatty acids, oleic acid represents 55 to 83% of total fatty acids (*Jacotot, 1997*).

Among polyunsaturated fatty acids, linoleic acid represents 3.5% to 21% of total fatty acids, and linolenic acid (0.7% - 0.9%). The main saturated fatty acids are stearic acid and palmitic acid, which represent 10 to 16% of the total fatty acids (*Roehly, 2000*).

- Unsaponifiable fractions

Unsaponifiable substances represent all the (natural) constituents that do not react with an alkaline hydroxide to give soaps and which, after saponification, remain soluble in conventional fat solvents. These substances represent 2% to 4% of the oil and are a complex mixture of compounds from various chemical families:

- Hydrocarbons;
- Tocopherols (vitamin E);
- Triterpenic and aliphatic alcohols;
- Sterols;
- Phenolic compounds (antioxidants);
- Chlorophylls and carotene.

Olive oil is characterized by its delicate and unique aroma. This very particular aroma is due to a whole range of components present in very low concentrations (*Benrachou, 2013*).

- Phenolic compounds

The phenolic compounds in the oil originate from the fruit and are transferred into the oil during the crushing process. This passage in oil already occurs at the tissue level, but the extraction process only reduces their concentration (*Brenes et al., 2002*).

### - Coloring pigments

The coloring of virgin olive oil is mainly due to the presence of colouring pigments belonging to the carotenoid and chlorophyll family (*Benrachou, 2013*).

### - Carotenoid pigments

The carotenoid pigments mainly present in olive oil is  $\beta$ -carotene (provitamin A). Its level varies from 0.3 to 3.7 mg / kg of oil. 2 mg of  $\beta$ - carotene are converted into 1mg of vitamin A (*Benrachou, 2013*).

### - Chlorophyll

This pigment, whose content may vary according to many factors, has a biologically active role in stimulating metabolism, stimulating cell growth, hematopoiesis (the formation of blood cells) and accelerating healing processes (*Nieves criado. and al., 2008*).

## ***1.2.5. Fraud detective of olive oil***

If we think that we are sure to know that 70% of the olive oils sold are not actually pure (and this includes extra virgin oils)! Olive oils were made which were blended with either canola oil or rapeseed oil, or mixed with a coarser, then chemically colored and fragrant olive version.

How to spot the difference?

- The taste test

Some tests are proposed to do at home to discover the olive oil, has not been tampered with. One of these tests is simply snacking. The problem is that fake olive oil has been selling for so long that even food experts can no longer tell the difference

An experiment carried out with several food experts. They tasted a wide range of olive oils. They approved the cheapest and most trafficked oil, and thought the purest oils were wrong!

- The refrigerator test

Another suggestion is to put olive oil in the refrigerator because the monounsaturated fats become heat disorders (even after a long time). So, if the olive oil does not change after being refrigerated for 2 hours, it is wrong!

This problem was mixed with untransformed oils. For this test to be cold. In the end, this test is not enough to eliminate oils purchased in stores.

### ***1.2.6. Olive oil oxidative stability***

Oxidative stability is an important parameter in evaluating the quality of oils and fats, as it gives a good estimation of their susceptibility to oxidative degeneration, the main cause of their alteration. The greater or lesser stability of an oil means the conservation or not of the so-called dynamic parameters during the useful life of the (*Barmak, 2011*).

The oxidative stability was measured by a Metrohm Rancimat model 743 (Herisau/Switzerland) as the reference analysis for all olive oil samples. Increasing the electric conductivity of the water was continually monitored while air (20 L/h) was bubbled into each oil (6 g) heated at different temperatures and their volatile oxidation products were collected in water.

The greater or lesser stability of an oil means the conservation or not of the so-called dynamic parameters during the useful life of the product. Decomposition of hydroperoxides has been established as being related to the number of double bonds in fatty acids. It is well-known that linoleate hydroperoxides decompose faster than oleate ones (*Frankel, 1962*). At the same time, any reaction preventing the propagation of peroxidation, or removing free radicals, plays an important role in the oxidation mechanism. Chain-breaking antioxidants, such as phenolic compounds, react with lipid radicals to form nonreactive radicals, stopping the propagation chain (*Simic, 1992*). In fact, these compounds are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (*Shahidi, 1992*)

### ***1.3. Qualitative characteristics of oil***

#### ***1.3.1. Determination of free acidity (acidity)***

Acidity is the percentage of free fatty acid conventionally expressed according to the oleic nature which is the dominant fatty acid.

It presents as an important parameter for the classification and marketing of olive oil and is a means of rapid control of the oil quality

This is a method that consists in determining the fatty acids released during the hydrolysis of the triglycerol chains which is carried out by dissolving an oil test sample in neutralized alcohol and then titrating with an aqueous solution of sodium hydroxyl. Phenolphthalein is used as a colored indicator.

#### ***1.3.2. Determination of the peroxide value***

As oxidation causes rancidity, or the quality deterioration of fats and oils, the peroxide value test is necessary to quantitatively determine if the fats and oils are oxidized, and the degree to which they are oxidized. The peroxide value test was identified by the International Olive Council as one of the critical measures to grade olive oil and its determined by the EU standard methods (*EU No 61/2011., 2011; EU No1348/2013., 2013*).

#### ***1.3.3. The specific coefficients of extinction at 232 nm and 268 nm ( $K_{232}$ and $K_{268}$ and $\Delta K$ ).***

All-natural fatty substances contain linoleic acid in more or less important quantities, the oxidation of a fatty substance leads to the formation of linoleic hydro-peroxides which absorb light in the vicinity of 232 nm. If the oxidation continues, the secondary oxidation products are formed, in particular unsaturated ketones which absorb light towards 268 nm.

The extinction at 232nm and 268nm of a crude fatty substance can be considered as no image of its oxidation state, the more its extinction at 232nm is strong plus it is peroxide and the one at 268nm is strong plus it is rich in products of the secondary oxidation.

The fat studied is dissolved in the required solvent, and then the extinction of the solution at the prescribed wavelength is determined relative to the pure solvent. Specific extinctions are calculated from the spectrophotometric readings.

#### ***1.3.4. Total phenols (TP) content***

The amount of phenolic compounds in VOOs is an important factor when we assess its quality. It contributes to the nutritional importance and benefit to human health of virgin olive oil and they are responsible for bitter taste and the antioxidant activity of the oil (*Garc and al, 2003; Kharazi and al, 2012*). Therefore, the content of phenolic compounds is an important parameter which determines the characteristics and quality of olive oil, and informing that natural phenolic compounds are able to improve the oxidation resistance (*Garcia and al, 2002; Kowalski and al, 2004*). The total amounts of phenolic compounds in olive oil depend on various factors such as cultivar, climate and irrigation, altitude and technological conditions during extraction (*Garcia and al, 2002; Garc and al, 2003*).

The rate of deterioration of linoleic acid decreases with the increase of concentration of anti-oxygen. Nevertheless, the addition of high concentrations induces the opposite effect, prooxidant effect (as in the case of high doses of  $\alpha$ -tocopherol) (*Cillard and al, 1980*). It is important to underline the temporary effect of the virtues of antioxidants; these molecules, in their active forms, gradually disappear from the middle. Antioxidants can be classified, according to their modes of action, as antioxidants primary and secondary antioxidants. They can be involved in all stages of the radical oxidative process, phenolic compounds are among the primary antioxidants that include synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). (*Kortenska et al, 2002*). These synthetic antioxidants are perceived as potentially toxic to consumers and therefore natural antioxidants are often preferred. Rosemary extracts (*Rosemarinus officinalis L.*) are used in manufacturing in the agro-food industries; their antioxidant activity consists in the inhibition of free radicals (*Basaga et al, 1997, Vareltzis et al, 1997*). The active ingredients of this natural extract are phenolic molecules and carnosic acids, rosemary and carnosol. These phenolic antioxidants are effective at low concentrations; they are often used, in manufacture, at doses lower than 0.01% (*Rajalakshmi, 1996*).

The content of total phenolic was determined spectrophotometrically using Folin–Ciocalteu's reagent according to the method described by (*Capannesi et al., 2000*).

#### **1.4. Oxidative stability**

Lipid oxidation is the main cause of the deterioration of containing fats (*Firestone, 1993*). The level of lipids oxidation can be measured by chemical and physical methods, as well as by stability tests measuring the stability of an oil under conditions favoring natural oxidation. Auto-oxidation includes the induction period and the oxidation period. This is the duration of the phase induction which gives information on the stability of a lipid. The longer the induction time, the longer the lipid is stable (*Markus, 1986*), the oxidative stability index principle OSI (is similar to that of the method with active oxygen) AOM (, but faster and more automated, it determines the stability oxidation of an oil by passing through a sample, subjected to a treatment controlled by a stream of air, which facilitates the rapid degradation of lipids volatile organic acids. The air stream captures the released volatile acids and solubilizes them in a tank filled with distilled water; the solubilized acids are dissociated into ions, thus causing a change in the conductivity of this water. The value of OSI is defined by time (in hours) required for the conductivity to reach a value predetermined (*Markus, 1986*).

The Rancimat is an automated device; it measures, in a very short time, the conductivity of low molecular weight of fatty acid released during lipid auto-oxidation heated to 100°C or more. The principle of determining conductivity is based on the measuring the resistance of the solution of the recovered volatile acids. This device can determine the lipid induction period and the anti-oxygen effect of the different antioxidants.

(*Luther et al, 2007; Romeu-nadalet et al, 2007*) used the Rancimat test to evaluate the relative stability of infant formulas and fish oils respectively. Nevertheless, several authors do not support the use of OSI for the evaluation of the oxidative stability of the oils (*Frankel, 1993*). suggested that "OSI and AOM "are inappropriate given the complexity of the oxidation mechanism of lipids; it changes significantly at very high temperatures. Levels oxidation become dependent on the available oxygen concentration because the solubility oxygen decreases at high temperatures. Oxidation control, including Determination of the peroxide content, conjugated dienes or the carbonyl levels of the oils is questionable or equivocal. In addition, side reactions become important and do not do not reflect the usual storage

temperatures; *Aeubli and Bruttel (1986)* compared the Rancimat method to that of active oxygen in determining the oxidative stability of fats and oils. they have concluded that these methods give the same results; but, the Rancimat method is more interesting because it does not require prior preparation of the sample. Because of its ease of use and reproducibility of the given results, the Rancimat test is very used to determine the stability of oils (*Mendez and al, 1996*).

## **1.5. Rancimat test**

### **1.5.1 Importance of the Rancimat**

The classic application of Rancimat Professional consists of to determine the oxidation stability of the oils and fats. Oxidation stability refers to the resistance oxidation of oils and fats.

The method of Rancimat accelerates the oxidation process by a high temperature and a continuous stream of air that act on the sample. The fatty acid molecules are then oxidized in the sample. The first oxidized products to develop are peroxides. After a certain time, the fatty acids are completely destroyed and secondary oxidation products appear.

These include, volatile organic acids with low molecular weight, such as acetic acid and formic acid will be driven by the current air in a second container, which contains distilled water and allows continuous measurement of conductivity.

The presence of volatile acids in the measuring vessel results in an increase in conductivity. The time that elapses until the appearance of the product of secondary reaction is the induction time, the period Induction or Oil Stability Index (OSI). This value characterizes the resistance of the sample to oxidation.

The longer the induction time, the longer the sample will be stable.

The induction time measured according to the Rancimat method is a standard parameter of quality control in manufacture, control of the goods upon their arrival or the treatment of oils and fats in the industry food. It is used in production plants and food processing. In addition to oils

and vegetable fats, the Rancimat Professional naturally allows determining the oxidation stability fats of animal or vegetable origin.

Foods usually contain antioxidants, which slow down the oxidative decomposition of oils or fats. These antioxidants can be original natural or artificially added.

The Rancimat Professional can also make a contribution valuable for studying the effectiveness of antioxidants current and future (*892 Professional Rancimat, Manual., 2002*).

### ***1.5.2 The instrument of the Rancimat***

A Rancimat model (Herisau, Switzerland) was used at several. For each time studied, eight samples were accommodated in the equipment and analysed simultaneously; the samples for all determinations were randomized on their position in the heating block. The OSI of the vegetable oils were automatically recorded and taken as the break point of the plotted curves (the intersection point of the two extrapolated parts of the curve). A kinetic rate constant was taken as the inverse of the OSI.

Moreover, Rancimat has established to be a fast and sufficient instrumental method for the evaluation of kinetic and thermodynamic studies of lipid oxidation of olive oil. Depending on the thermodynamic factors, the nature of the oxidation reaction should prove that it is an endothermic and non-spontaneous process after kinetic analysis.

#### ***Advantages of the device:***

- Control all functions of the device from the computer
- Overview of the status of each measurement position on the device screen

Many other methods besides the Rancimat test; we can cite the pressure differential scanning calorimetry (PDSC), differential scanning calorimetry (DSC) techniques and Oxytest technics those technics gives results close to the Rancimat test.

## 1.6. Methodology

To fulfil the aims of this study, *three tasks* will be performed. In *the first* one it will be the collect of the samples so we can say that the study in this thesis was carried out on several samples of olive oil

The samples were stored in the bottles in the dark. These oils have undergone physicochemical analyzes in this study which will be presented in this work.

In *the second task* we will classify the samples per their Quality which is defined as the combination of attributes or characteristics of a product that have significance in determining the degree of acceptability of that product by the user. Olive oil quality may be defined from commercial, nutritional or organoleptic perspectives. The nutritional value of EVOO (extra virgin olive oil) originates from high levels of oleic acid content and minor components, such as phenol compounds, whereas the aroma is strongly influenced by volatile compounds. Nutritional value and unique pleasant flavor promotes consumption demand and price of olive oil in comparison with other edible oils. In order to fulfil the expectations of consumers, good quality control of olive oil should be assured in the course of production and storage line. The quality of olive oils is maintained in terms of measurement of analytical parameters for which certain limit values are set.

In the *third task* we will calculate the kinetic parameters and thermodynamic parameters such as temperature coefficients activation energies and frequency factors and also the entropy; enthalpy and Gibbs free energy.



## **2. Materials and methods**

## ***2.1. Olive oil samples***

The aim of this investigation has been to study three types of virgin olive oil according to their total content of phenols and for each laboratory we have done the test 5 times for each type;

Our olive oil is from “Macedo de cavaleiros mil olimontes” olive oil from year 2018

The studied olive oil was obtained from Olimontes mill (Macedo de Cavaleiros, Portugal).

Three types of olive oil were supplied, namely:

- An Arbequina(ARB) monovarietal olive oil, which was expected to have a low total phenols content;
- A blend olive oil produced from cvs Madural (MAD), Cobrançosa (COB) and Verdeal Transmontana (VT); expected to have a medium total phenols content;
- A COB monovarietal olive oil, which was expected to have a high total phenols content;

## ***2.2. Determination of free acidity***

### **a. Definition**

Acidity is the percentage of free fatty acid conventionally expressed according to the oleic nature which is the dominant fatty acid.

It presents an important parameter for the classification and marketing of olive oil and is a means of rapid control of the quality of the oil.

This is a method that consists in determining the fatty acids released during the hydrolysis of the triglycerol chains which is carried out by dissolving an oil test sample in neutralized alcohol and then titrating with an aqueous solution of sodium hydroxyl. Phenolphthalein is used as a colored indicator.

## **b. Operating mode**

In a small bottle: 5g of olive oil + 50 mL (diethyl eter + ethanol 1: 1, v/v) and add 3 drops of phenolphthalein than shake the mixture well using a vortex. Titrate with aqueous solution of NaOH (0.3546N) until the color change and we get a light pink color.

The acidity expressed as a percentage by weight of oleic acid will be equal to:

Equation 1 free acidity equation

$$FA\% = \frac{N*V*10^{-1}*M}{m} \quad (1)$$

*N*: the normality of the standard solution of NaOH (= 0.3546N)

*V*: volume in ml of the NaOH solution

*M*: mass of oleic acid (= 282 g/mol)

*m*: mass in grams of the test sample (= 5g)

## **2.3. Determination of peroxide value**

### **a. Definition**

This index is sought to evaluate the conservation state of a fat during storage, it is the number of hydro-peroxides expressed in milliequivalent of oxygen per kg of fat present in the oil. In fact, the fatty substances can be oxidized in the presence of oxygen and certain favorable factors. This oxidation, called auto-oxidation or aldehyde rancidity, leads initially to the formation of peroxides by fixing one mole of oxygen on the carbon with respect to an ethylenic bond of the unsaturated fatty acids constituting the glyceride. The test portion in solution in an acetic acid and chloroform mixture is treated with a potassium iodide solution. The iodine released during oxidation is titrated with sodium thiosulfate solution.

### **b. Operating mode**

In an Erlenmeyer flask :

1.2g of olive oil + 10mL of chloroform + 15 mL of glacial acetic acid + 1mL of a saturated solution of potassium iodine KI. Cover and shake the mixture for 1 minute and let stand for

5min in the dark dilute the solution with 75ml of distilled water + starch solution (0.1g of starch in 10mL of distilled water) as a colored indicator => solution of navy-blue color

Titrate the liberated iodine with sodium thiosulphate solution until the solution is completely incolored.

Equation 2 peroxide value equation

$$PV(mEq O_2/kg) = \frac{V*2}{m} \quad (2)$$

*V: Thiosulfate volume added (mL)*

*m: mass of oil (g)*

*2: dilution*

## **2.4. Specific coefficients of extinction (232nm and 268nm, $K_{232}$ , $K_{268}$ , $\Delta K$ )**

### **a. Definition**

This spectrophotometric examination in the ultraviolet can provide indications on the quality of a fat (**Benabid, 2009**). The extinction coefficient at 268 nm is a good indicator of the peroxide content of the oil.

The determination of the absorbance at 232 nm and at around 268 nm allows the detection of the oxidation products of unsaturated fatty acids, when they have a conjugated diene structure (C18: 2 linoleic hydro-peroxide), and by-products oxidation having a triene structure (**Benabid, 2009**), in particular ketones and diketones, which absorb light at around 268nm.

The principle is to dissolve the fat in the required solvent, and then determines the extinction coefficient  $\epsilon^{1\%}$  of the solution at the prescribed wavelength, by supplying the pure solvent.

Specific extinctions are determined from the recorded spectrophotometric data (**Bouhadjra, 2011**).

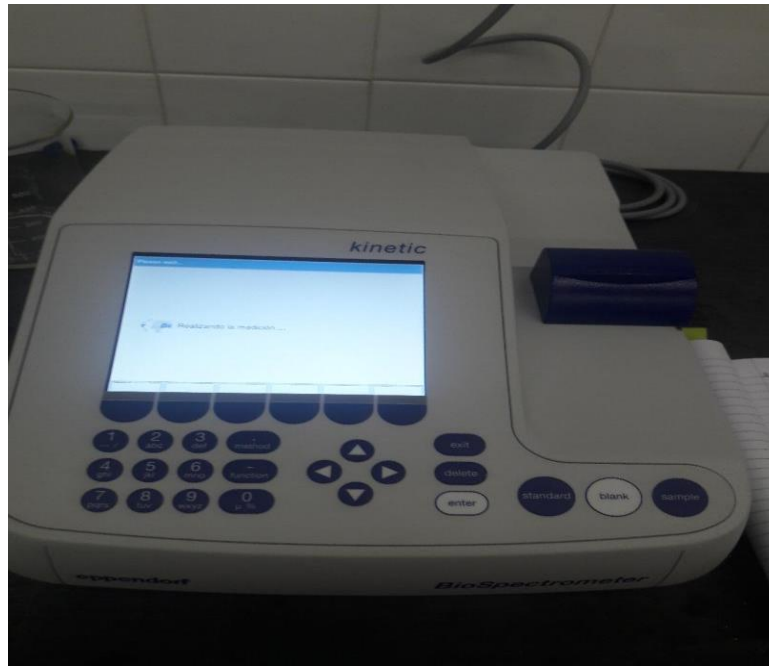


Figure 1. Spectrophotometry machine

## b. Operating mode

The  $K_{232}$  and  $K_{268}$  specific extinctions are calculated as follows:

- 0.6 g of olive oil and 10mL of cyclohexane were putted in a tube (our mother solution).
- After homogenization, 0.5 mL of the mother solution was taken in a tube and adjusted it to 10 mL with isooctane to measure the extinctions coefficients at the wavelengths 232 nm and 268nm. We take 2ml of the mother solution in another tube and adjusted up to 10ml with isooctane. With respect to ( $\Delta K$ ), the wavelength-specific extinctions coefficients were measured at 232 and 268nm (isooctane is used as blanc)

Equation 3 The extinction coefficient at 232 nm

$$K_{232} = \frac{A_{232}}{P \cdot 4} \quad (3)$$

Equation 4 The extinction coefficient at 268 nm

$$K_{268} = \frac{A_{268}}{P \cdot 4} \quad (4)$$

Where  $P$  is the weight of the test sample

*A is the absorbance at 232nm or at 268nm*

*4 is the dilution factor*

Equation 5 Temperature Dependence

$$\Delta K = K_{268} - \frac{K_{264} + K_{270}}{2} \quad (5)$$

## **2.5. Total phenols content**

### **a. Definition**

Phenolic compounds are one of the most important classes of molecules in the unsaponifiable fraction of olive oil. These compounds are of great interest because of their antioxidant activity, which has several beneficial effects on human health a wide range of polyphenols has been identified in olive oil and an antioxidant is defined as "any substance capable of delaying or slowing down the rancidity (action of O<sub>2</sub> on unsaturated fatty acid UFA), discoloration or appearance of flavors oxidation" thus, the antioxidant increases the shelf life of a food and reduces vitamin or essential fatty acid EFA losses.

### **b. Operating mode**

The total phenolic contents were determined according to (*Capannesi et al; 2000*) with some modifications. A mass of 2.5 g of olive oil was diluted with hexane and then extracted with 2.5 mL of methanol/water (80:20 v/v). The mixture was then centrifuged (5 min at 5000 rpm). After, to 1 mL of the supernatant, 1 mL of Folin-Ciocalteu reagent, 1 mL of Na<sub>2</sub>CO<sub>3</sub> solution (7.5%), and 7 mL of distilled water were added, after homogenization, the mixture was stored overnight and spectrophotometrically analyzed ( $\lambda=765\text{nm}$ ).

Results were expressed as mg of caffeic acid equivalents per kg of oil.

## 2.6. *Oxidative stability*

### a. **Definition**

Oxidative stability of each oil samples was measured under accelerated conditions. Experiments were conducted on Rancimat 892 apparatus (Metrohm, Herisau, Switzerland) using the Rancimat method at 120, 130,140,150 and 160° C. Weighing the oil samples is of great significance since amount of the sample affects the reaction with oxygen (*Pullen, 2012*). Induction time (IT) was used to state the stability of the oils. The OSIs of the different oil sample were automatically recorded and taken as the break point of the plotted curves, the intersection point of the two extrapolated parts of the curve.

### b. **Operation mode**

The oxidative stability of the olive oil was evaluated using a Metrohm Rancimat apparatus model 743(Metrohm, Switzerland) capable of operating over a temperature range of 50–200°C was used for induction period determination of olive oil samples.

Three olive oil samples were analyzed in the equipment. For oxidative stability measurement, each oil sample ( $3.0\pm 0.1$  g) was weighted in vessel glassware. The volatile oxidation products formed under forced conditions are transported by the air stream into the conductometric cell, and detected by continuously measuring the water electrical conductivity ( $\mu\text{S cm}^{-1}$ ) over time (h). The conductimetry cells are filled with diluted water-up to 60 mL. Air is flown through the heated oil at different rates, depending on the temperature.

The oxidative stability is expressed corresponding to the time (h) at the intersection point between the horizontal (conductivity,  $\mu\text{S min}^{-1}$ ) and vertical (time, h) tangents of the fitted exponential oxidation curves. At this break point the water conductivity increased overtime because of the fat oxidation.

Although, this equipment (Model 743 Metrohm) presents a cleaner system of procedure, the glassware is rigorously cleaned between each run to avoid any contamination that would catalyze the peroxidation. The tubes were rinsed with acetone, and then cleaned with detergent and hot running water. After that, they were rinsed with running water and distilled water. Clean glassware is thoroughly dried in an oven.

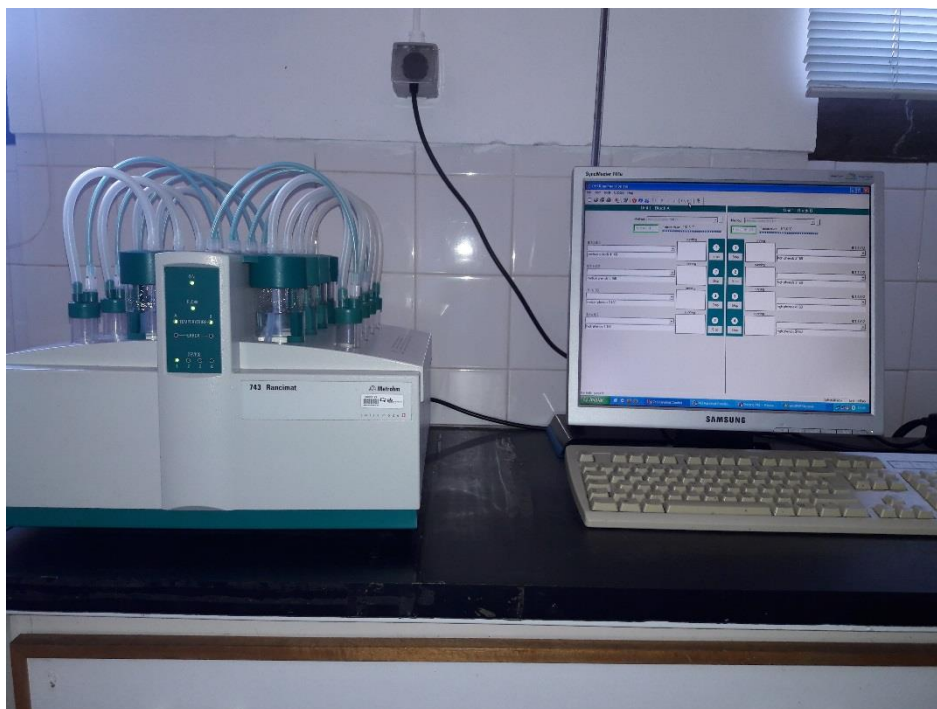


Figure 2. Rancimat equipment (Herisau, Switzerland Metrohm)

## 2.7. Kinetic parameters

The speed of chemical reactions depends strongly on the temperature. Experimental studies are therefore generally conducted at a constant temperature.

To study the influence of temperature, several isothermal studies were carried out at different temperatures.

- Determination of activation Energy and pre-exponential factor

First of all, it must be ensured that the reaction under consideration has an Arrhenius behavior.

For this, we can see that it is necessary to have several values of the speed coefficient determined at different temperatures over a fairly wide range. The verification of the alignment of the points  $(\ln k, \frac{1}{T})$  makes it possible to determine the value of the activation energy from the direction coefficient of the straight line passing through these points and the preexponential factor from its ordinate at the origin.

If we are certain that the reaction has an Arrhenius behavior, we can determine  $E_a$  from two constant  $k$  and  $T$ .

Equation 6 rate constant equation

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (6)$$

*k* is rate constant

*E<sub>a</sub>* is the activation energy of the reaction

*A* is the frequency factor

*R* is the constant of perfect gases (8.314 J.mol<sup>-1</sup>. K<sup>-1</sup>) *T* the temperature in Celsius

temperature coefficients (*T*<sub>Coef</sub>, K<sup>-1</sup>) can be determined from the slopes of the curves generated by plotting ln(*k*) vs. the absolute temperature (*T*, K):

Equation 7 the plot of ln(*k*) vs. the absolute temperature (*T*, K)

$$\ln(OSI) = a(T) + b \quad (7)$$

Where *a* and *b* are the equation parameters the intercept and slope from the regression equation line

Activation energies (*E<sub>a</sub>*, kJ/mol) and pre-exponential or frequency factors (*A*, h<sup>-1</sup>) were determined from the slopes and intercepts, respectively, of the lines generated by plotting ln(*k*) vs. 1/*T* using the Arrhenius equation:

Equation 8 the Arrhenius equation

$$\ln(k) = \ln(A) - \left(\frac{E_a}{RT}\right) \quad (8)$$

- The use of Arrhenius relationship

Once the values of the activation Energy and preexponential factor are known, it can be used to calculate the value of the velocity coefficient at another temperature. However, caution must be exercised and not extrapolated to temperatures too far from the range in which the reaction was verified to follow Arrhenius's law.

as *Q*<sub>10</sub> number, was calculated from the slopes of the lines.

Equation 9 *Q*<sub>10</sub> number equation

$$Q_{10} = 10^{-10Tc} \quad (9)$$

- The activation energy  $E_a$

Activation energy is the minimum amount of energy required to initiate a chemical reaction, whether endothermic or exothermic.

In order for a chemical reaction to proceed, the reagent molecules must collide. Indeed, according to the theory of collisions, certain conditions are necessary for the course of a reaction. The collision of the reagent molecules must be efficient and not elastic, which makes it possible to form the activated complex. It must therefore be performed with a minimum of energy, which is called the activation energy.

The equation to determine the activation energy is:

Equation 10 free energy equation

$$E_a = \frac{R \left( \ln \frac{k_2}{k_1} \right)}{\left( \frac{1}{T_2} - \frac{1}{T_1} \right)} \quad (10)$$

- Enthalpy and entropy

The enthalpy of a system is therefore a measure of the energy of the system available under form of heat at constant pressure. A process that lowers the enthalpy of a system will be called exothermic ( $\Delta H < 0$ ). On the contrary, a process that leads to an increase in the enthalpy of the system will be called endothermic ( $\Delta H > 0$ ).

In the other hand an entropy, in thermodynamics and fluid mechanics, calculates the ratio of the amount of heat exchanged by a system at a fixed temperature and this variation indicated by a state function with a unit of joule per Kelvin (J / K). This state function, noted  $\Delta S$ , is called entropy. Entropy, from a statistical point of view, measures the degree of disorder of a system at the microscopic level. The higher the entropy of the system, the less orderly its elements are, the less interrelated they are, and the less they become capable of producing mechanical effects, and therefore the greater the share of unused or used energy.

Equation 11 the activated complex theory equation

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{kb}{h}\right) + \left(\frac{\Delta S^{++}}{R}\right) - \left(\frac{\Delta H^{++}}{RT}\right) \quad (11)$$

Where  $k_b$  is the Boltzmann constant ( $1.3806586 \times 10^{-23} \text{ J/K}$ ) and  $h$  is the Planck's constant ( $6.62607556 \times 10^{-34} \text{ J s}$ ). From the slopes and intercepts of the lines,  $\Delta H$  and  $\Delta S$  were calculated

We have as an equation  $y = ax + b$  and referring to the equation of the activated complex theory we can say that:

Equation 12 the intercept of the activated complex theory

$$b = \ln 10 \left(\frac{kb}{h}\right) + \left(\frac{\Delta S^{++}}{R}\right) \quad (12)$$

As a result, we have:

Equation 13 entropy equation

$$\Delta S^{++} = \left(b - \ln \frac{kb}{h}\right) * (R) \quad (13)$$

For a

Equation 14 the slope of the activated complex theory

$$a = -\frac{\Delta H^{++}}{R} \quad (14)$$

So, as a result, we have

Equation 15 enthalpy equation

$$\Delta H^{++} = -a * R \quad (15)$$

Equation 16 Gibbs free energy

$$\Delta G^{++} = \Delta H^{++} - T * \Delta S^{++} \quad (16)$$

## 2.8. *Statistical analysis*

The described assays were performed in duplicate and the results were expressed as mean  $\pm$  standard deviation (SD). The data for the evaluation of the olive oil, in the several assays, were analyzed through the t-student test, with the purpose of determining the significant differences between 2 samples, with p-value = 0,05. Fitting procedures, coefficient estimates and statistical calculations were achieved as previously described by other authors (*Prieto, 2014*). In brief, a) the parameters determination was accomplished using the quasi-Newton algorithm (least-square) by running the integrated macro 'Solver' in Microsoft Excel minimizing the differences between observed and predicted values) the coefficient significance was evaluated using the 'SolverAid' macro to determine their intervals ( $\alpha= 0.05$ ); and c) the model consistency was proved by means of several statistical criteria) the Fisher F-test ( $\alpha= 0.05$ ) was used to assess the adequacy of the models to describe the observed data) the 'SolverStat' macro was used for the assessment of parameter and model prediction uncertainties (*Murado, 2013*) the  $R^2$  was interpreted as the proportion of variability of the dependent variable explained by the model.

# **3. Results and discussion**

### 3.1. Physicochemical results

The **Table 1** is a comparative research between the obtained results of the present study and several published study. it shows the olive cultivar and the olive oil group and its classification and their physicochemical results which are free acidity, peroxide values and specific coefficients of extinction.

Table 1. Physicochemical results of several types of olive oil

	Olive cultivar	Olive oil group	FA (%)	PV(mEq O <sub>2</sub> /kg)	K <sub>232</sub>	K <sub>268</sub>	Classification*
<b>This work</b>	<b>Arbequina cv</b>	<b>Low phenols</b>	0.17±0.06	9.42±0.49	1.9±0.05	0.19±0.01	EVOO
	<b>Blend olive</b>	<b>Medium phenols</b>	0.21±0.00	6.93±0.48	1.19±0.04	0.15±0.08	EVOO
	<b>Cobrançosa cv</b>	<b>High phenols</b>	0.23±0.03	3.6±0.49	1.17±0.08	0.17±0.01	EVOO

\* Olive oil quality grade classification based on the physicochemical levels [1–3]: EVOO (simultaneously: FA ≤ 0.8% oleic acid, PV ≤ 20 mEq O<sub>2</sub>/kg, K<sub>232</sub> ≤ 2.50, K<sub>270</sub> ≤ 0.22,); VOO (simultaneously: FA ≤ 2.0% oleic acid, PV ≤ 20 mEq O<sub>2</sub>/kg, K<sub>232</sub> ≤ 2.60, K<sub>268</sub> ≤ 0.25, and, ΔK ≤ 0.01) or LOO (for the other cases).

#### 3.1.1. Free acidity

The results of the free acidity contained in our three samples were done 5 times (low, medium and high phenols content) respectively are summarized in the **table 1**.

According to the standards of the International Olive Council, 2015 on free acidity (**Annex 1**). It is noted that the free acidity of low, medium and high phenols olive oil are within the limits established, which is less than 0.8% and allows them to be classified as extra virgin olive oil.

The determination of the acidity index is given by the free acidity how allows to control the level of hydrolytic, enzymatic or chemical degradation of the fatty acid chains triglycerides. This is the origin of free fatty acids and partial glycerides (mono and diglycerides).

The index of the extra virgin olive oil is linked to the sanitary freshness of the olive, to the mastery of the process technology implemented for the conservation storage and transformation of the raw material and the degree of maturity of the fruits (*Sekour, 2012*).

### ***3.1.2. Peroxide value***

The results recorded in **Table 1 and table 2** show that the values obtained from **IOC (2015)** standards that recommend a lower peroxide index or equal to 20 meq O<sub>2</sub> / kg.

It is observed that the peroxide index (IP) in the sample low phenols, estimated at 9.42 meqO<sub>2</sub>/kg, seems higher compare to the remains samples. Values of IP thus encountered ranging from 3.60 meq O<sub>2</sub> / kg for high phenols to 6.93 meq O<sub>2</sub> / kg for medium phenols sample.

The peroxide index (IP) it estimates the state of autoxidation of the oil, it's a slow mechanism but inescapable. Indeed, the fatty substances can oxidize in the presence of oxygen and certain favorable factors (high temperature, water, enzyme, trace of metals Cu, Fe ...). This autoxidation or rancidity aldehyde leads in a first step to the formation of peroxides (or hydroperoxides) that break down subsequently carbonyl derivatives aldehydes and hydrocetones (responsible for the odor rancid) and various oxygenated products (alcohols, acids, etc.) (*Tanouti et al, 2011*).

The determination of the peroxide index is the most appropriate method for measurement of these peroxide compounds. For the three samples of olive oil analyzed, it was found a peroxide value less than 20 meq O<sub>2</sub> / kg, these values remain low and, in the standards, set by the IOC for olive oil. These low IP values are rising that the oil was extracted quickly after the olive harvest and that it was stored in good conditions. It suggests that the oil will not oxidize prematurely and will keep over time. It should be noted that the IP increases with the maturity of the olives, and especially following a thermal shock, following a freeze (*French Association Interprofessional Olive (AFIDOL), 2014*) or a defective manufacturing process, inadequate or prolonged storage is also one of the causes increases of this IP parameter (*Tanouti et al, 2011, Meftah et al, 2014*).

**Table 2.** Results of the peroxide value

<b>Sample</b>	<b>Average</b>	<b>SD</b>
<b>Arbequina olive oil</b>	9.42	0.49
<b>Blend olive oil</b>	6.93	0.48
<b>Cobrançosa olive oil</b>	3.60	0.49

Based on the IOC all the olive oils could be determined as Extra Virgin Olive Oil

### **3.1.3. Specific coefficients of extinction (232 nm and 268 nm, $K_{232}$ , $K_{268}$ , $\Delta K$ )**

PV values  $\leq 20$  meq  $O_2$  / g of oil do not always mean the absence of the oxidation phenomenon. The determination of the extinction coefficients ( $K_{232}$ ,  $K_{268}$ ), as **table 3** provides information on the presence or absence of secondary oxidation products in the oil,

The extinction coefficient at 232 nm ( $K_{232}$ ) and 268 nm ( $K_{268}$ ) were calculated from the respective absorbance values using equations (2) and (3):

Enthalpies ( $\Delta H^{++}$ ) and entropies ( $\Delta S^{++}$ ) of activation were obtained by regressing  $\log(k/T)$  versus  $(1/T)$  ( $K^{-1}$ ) via the equation derived from the activated complex theory:

The hydro-peroxides of the first stages of oxidation absorb at 232 nm, then that secondary oxidation products such as unsaturated ketones-diketones absorb in the vicinity of 268 nm.

Absorbance in the ultraviolet is a way evaluation of the state of conservation of the oil; it is also an indicator of the extraction method and the oxidation by over-exposure of the oil to air during crushing (*Boulfane et al, 2015*).The values of the specific  $K_{268}$  ultraviolet extinctions coefficients obtained for the samples indicate that they didn't exceed the limits set by the *IOC (2015)* which are less than or equal to **0.25** for of the extinction coefficient  $K_{232}$  varies from a sample to another but remains comparable to the standards established by the standards established by *IOC (2015)* equal to 2.60. the samples present results of  $K_{232}$  and  $K_{268}$  that respect the values recommended by the IOC.

Comparing our results with the results done before like that (*Gharbi, 2000; TekayaImen, 2005 and the Official Journal of the Olive Council International*)'s results who have worked on extra virgin olive oil and they find results not far from ours we can see it comparing the free acidity that they did not exceed the limit annocated by International Olive Oil Council. and it's the same for the peroxide value and the Specific coefficients of extinction ( $K_{232}$ ,  $K_{268}$ ).

Table 3 results of specific extinction coefficient

<b>SAMPLE</b>	<b>K232</b>	<b>K268</b>	<b>ΔK</b>
<b>L1</b>	0.574±0.05	0.252±0.01	-0.0016
<b>L2</b>	0.595±0.05	0.257±0.01	-0.0007
<b>L3</b>	0.598±0.05	0.233±0.01	-0.0017
<b>L4</b>	0.60±0.05	0.254±0.01	-0.0019
<b>L5</b>	0.607±0.05	0.226±0.01	-0.0017
<b>M1</b>	0.361±0.04	0.176±0.08	-.0046
<b>M2</b>	0.342±0.04	0.206±0.08	-0.005
<b>M3</b>	0.357±0.04	0.357±0.08	0.1189
<b>M4</b>	0.366±0.04	0.366±0.08	0.1153
<b>M5</b>	0.379±0.04	0.379±0.08	0.1343
<b>H1</b>	0.680±0.08	0.235±0.01	-0.003
<b>H2</b>	0.633±0.08	0.226±0.01	-0.0044
<b>H3</b>	0.738±0.08	0.220±0.01	-0.0038
<b>H4</b>	0.757±0.08	0.195±0.01	-0.0038
<b>H5</b>	0.722±0.08	0.219±0.01	-0.0039

### 3.1.4. Total phenols content

The oxidative stability of the oil decreases slightly as the fruits advance in the maturation. This regression is generally attributed to different metabolic responses varieties and decreasing the polyphenol content during ripening (*Beltran et al, 2005*). The **table 4** shows the results of the colorimetric assay of total polyphenols expressed in milligrams. It provides that the three olive oils analyzed contain appreciable quantities of phenolic compounds in different quantities.

Table 4. Total phenol content results

Sample	Mean±sd (mg CAE/kg)	Stander deviation
<b>Arbequina monovarietal olive oil</b>	86±10	10.4
<b>Blend olive oil</b>	113±7	7
<b>Cobrançosa monovarietal olive oil</b>	143±4,1	4,1

From this result we can constate that the absorption at 725 gives us a variation from 86.207 mg/Kg as a low phenol content to 143.851mg/Kg as a highest result and for the medium we have 113.084mg/Kg

### 3.2. Oxidative stability: (Rancimat test)

Oxidation resistance, often known as the Rancimat test, is an important parameter for the characterization of olive oil. This test allows determine the time required for the olive oil studied to the begin of rancidity symptoms due to fatty acid degradation unsaturated by the oxygen of the air. **Table 5** presents the results of this test on oil olive (per hour).

**Table 5.** Olive oils oxidative stability (OS, h) at different temperatures, measured using the Rancimat test

Temperature	Olive oils oxidative stability (OS, h)		
	Low TP content <sup>†</sup>	Medium TP content <sup>†</sup>	High TP content <sup>†</sup>
120°C	7.09± 0.22	12.16± 0.66	13.60 ±0.70
130°C	3.39 ±0.09	5.66 ±0.06	6.12± 0.04
140°C	2.01 ±0.05	3.11± 0.06	3.24± 0.07
150°C	1.22 ±0.06	1.77 ±0.03	1.86± 0.06
160°C	0.89 ±0.02	1.10± 0.01	1.17 ±0.04

<sup>†</sup>TP: total phenols; Low TP content: cv Arbequina olive oils with a mean TP content of 86±10 mg CAE/kg olive oil; Medium TP content: blend olive oils of cvs Cobrançosa, Madural and Verdeal Transmontana, with a mean TP content of 113±7 mg CAE/kg olive oil; High TP content: cv Cobrançosa olive oils with a mean TP content of 144±4 mg CAE/kg olive oil

The results showed that olive oil with high content of phenols has the highest oxidative resistance significant (13.60 h) at 120 °C and is therefore the most stable during storage as a conclusion also that at those temperatures studied the olive oil is more stable than the others with high phenol content.

The degree of high unsaturation in fatty acids offers a great susceptibility to the formation of radicals' free radicals that accentuate the oxidation process (*Martin-Polvillo et al, 2004*).

The Phenolic compounds are the primary antioxidants that inhibit the thermal oxidation of olive oil, they act as chain breakers by giving a hydrogen to the radical alkyl-peroxyl formed during the onset of lipid oxidation.

The measurement of induction time under standard conditions is generally used as an index of antioxidant efficacy (*Carrasco-Pancorbo et al, 2007*).

Oxidative stability does not only depend on the antioxidant pool, it is affected by the prooxidant compounds that may exist in the oil (*Bester et al, 2008*).

### ***3.3. Kinetic parameters***

For each temperature, we try to establish the speed law and determine the speed coefficient. In general, the shape of the velocity law does not change with temperature, only the value of the coefficient of velocity varies. Then we try to establish the relation between the value of the coefficient of speed and the temperature.

#### ***3.3.1. Arrhenius law***

The quantitative study of the variation of the coefficient of velocity with the temperature allowed Svante Arrhenius to propose in 1889 an equation which bears his name:

The rancimat method allowed us to determine the oxidation stability index figured in the **annex 5** in each temperature (120, 130, 140, 150 and 160°C) For each sample of our olive oil we calculated the kinetic parameters, as we worked with three types of olive oil the low, medium and high phenol content, for each one we did a specific where calculated from the regression equation regulation line the intercept and slope:

- **The low phenols content**

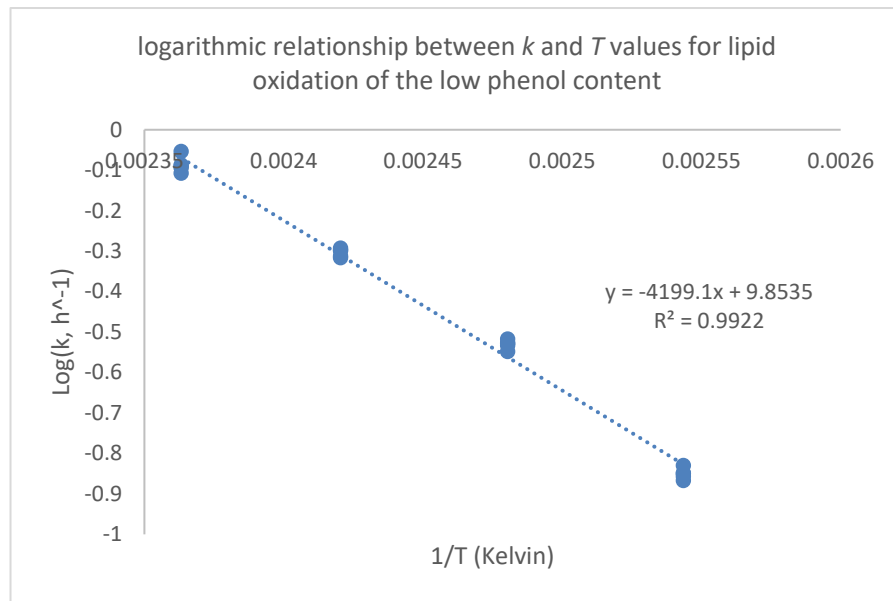


Figure 3. Logarithmic relationship between  $k$  and  $T$  values for lipid oxidation for the low phenol content

- **Medium phenol content**

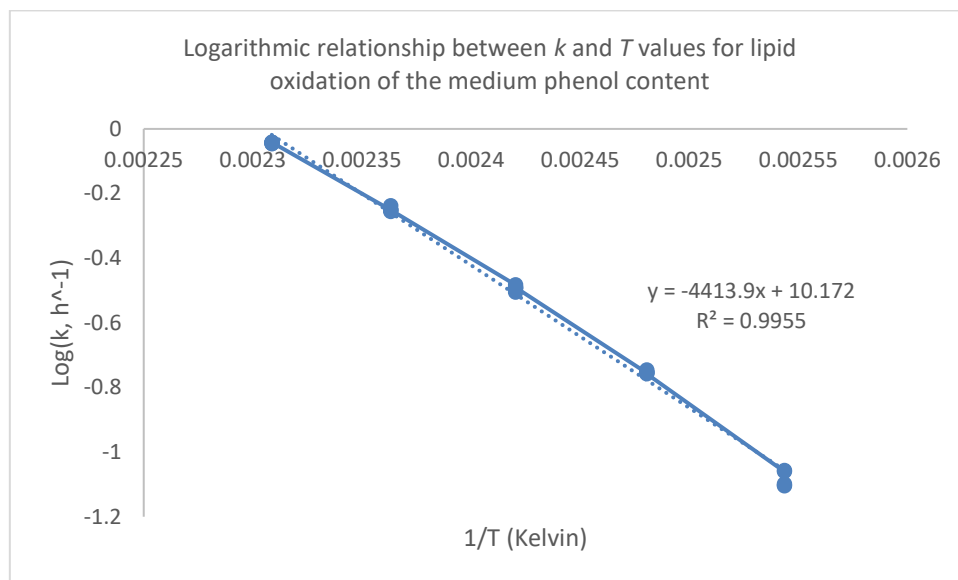


Figure 4. Logarithmic relationship between  $k$  and  $T$  values for lipid oxidation of the medium phenol content

- **High phenols content**

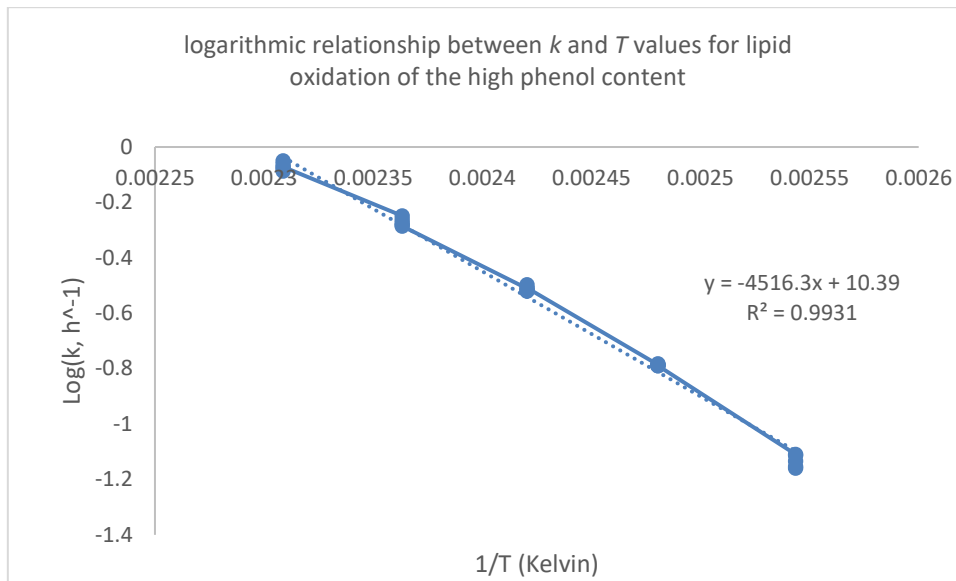


Figure 5. Logarithmic relationship between  $k$  and  $T$  values for lipid oxidation of the high phenol content

the Logarithmic relationship between  $k$  and  $T$  values for lipid oxidation of the olive oil allows to determine the kinetic parameters due to Excel

The table here below summarize the calculated kinetic parameters for the three investigated olive oil:

Table 6. Results of kinetic parameters

	Arbequina olive oil	Blend olive oil	Cobrançosa olive oil
Q10	1.68	1.81	1.83
Tc	-0.0224 °C <sup>-1</sup>	-0.02586 °C <sup>-1</sup>	-0.02645 °C <sup>-1</sup>

After calculating the  $k$  and the  $T$  we can calculate the activation energy  $E_a$  from the curve with excel data analysis (Regression) for each temperature (120,130,140,150 and 160°C);

The activation energy is the energy that must be brought to the chemical system for the reaction to take place.

**Table 6** provides the regression parameters for the Arrhenius relationships between the reaction rate constant  $k$  and the temperature for the four virgin olive oils studied ( $\ln(k) = \ln(A) - (E_a/R) \cdot (1/T)$ ). Using these regression parameters, frequency factors ( $A$ ,  $\text{h}^{-1}$ ), activation energies ( $E_a$ ,  $\text{kJ/mol}$ ), and Q10 numbers for the formation reaction of the secondary oxidation products under the Rancimat test conditions (volatile acids, mostly formic acids, with lower amounts of acetic acid, propionic acid, and other acids) were calculated (*DeMan and al., 1987*).

Employing  $E_a$ , which is the least amount of energy that must be overcome in order for a chemical reaction to occur, is another way to express the dependence of the rate of polyunsaturation present in the olive oil, so that the high contents of phenol content would increase  $E_a$  of lipid oxidation.

- **low phenol content**

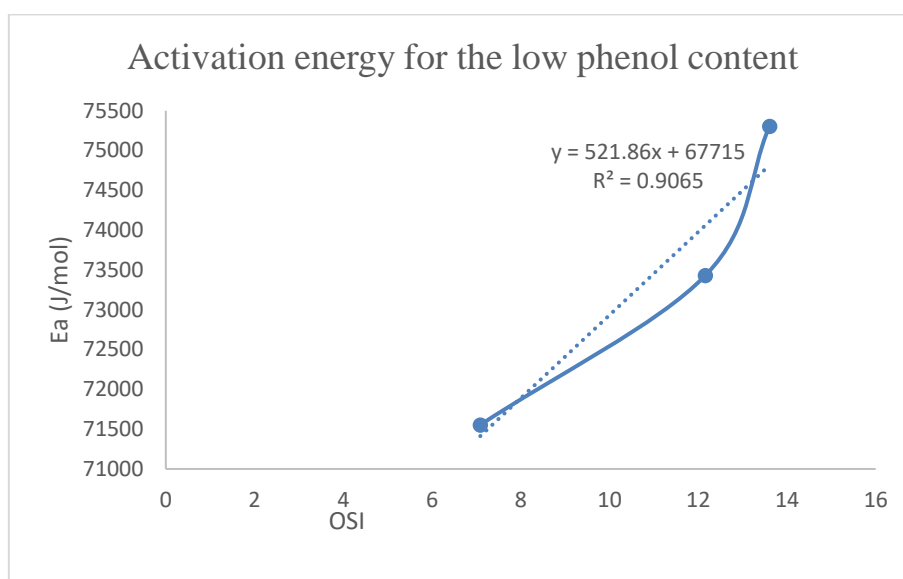


Figure 6. Activation energy  $E_a$  for the low phenol content

- **medium phenol content**

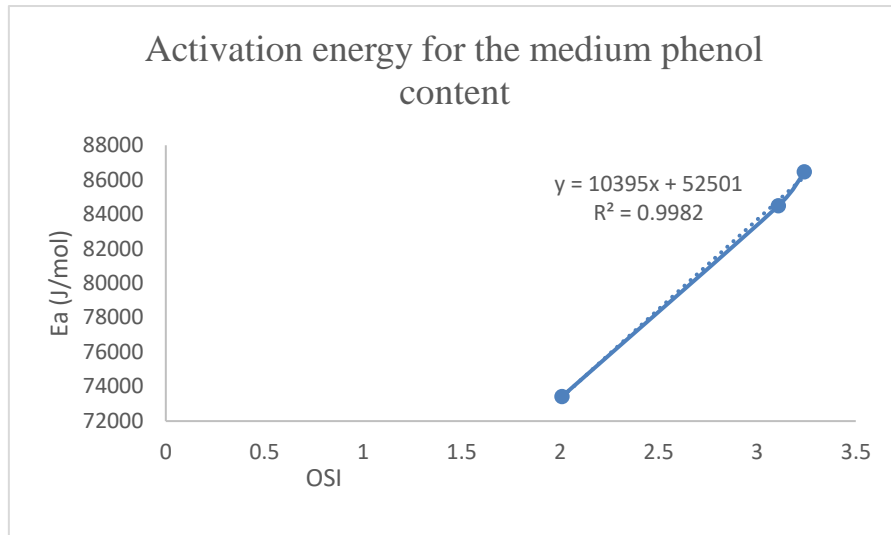


Figure 7. Activation energy  $E_a$  for the medium phenol content

- **High phenol content**

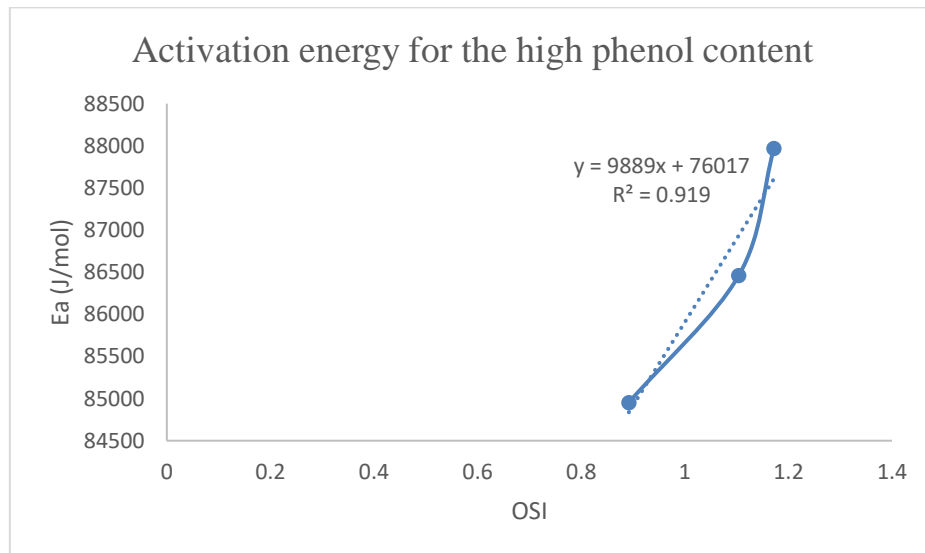


Figure 8. Activation energy  $E_a$  for high phenol content

The calculation of the activation energy is given in the table below:

Table 7. Activation energy values

	Arbequina olive oil	Blend olive oil	Cobrançosa olive oil	P-value <sup>1</sup>
E <sub>a</sub> (J/mol)	73429±1876 <sup>b</sup>	84497±1190 <sup>a</sup>	86460±1506 <sup>a</sup>	0.0001
A(h-1)	7.14E+09	1.49E+10	2.45E+10	

<sup>1</sup>P-value for one-way ANOVA (values lower than 0.05 mean a that there is a statistical significant difference at a 5% level); different lower-case letters mean significant statistical differences at a 5% significance level for the t-Student test

As can be seen in the previous figures (**figure 9, figure 10, figure 11**), the curves obtained are a straight line with a correlation coefficient close to 1 ( $R^2 = 0.9065, 0.9982, 0.9190$ ) respectively. Using this representation, the activation energy  $E_a$  can therefore be determined it is respectively (80383, 84495, 86474 J/mol). It increased with the concentration of low, medium and high phenol content due to ANOVA method.

This result is in the same order of magnitude than that obtained in similar work done on olive oil. During the oxidation of the olive oil samples, the  $E_a$  significantly varied from 8935 J/mol to 11316 J/mol (*Farhoosh, et al; Al 2014*).

In the other hand, the comparison of the studies done by *Mansooreh., 2017* for the kinetic parameters derived with Arrhenius equation and activated complex theory of Zard and Roghani oils gives those  $E_a$  values respectively 94300 and 106800 J/mol. This result is quite similar to the done study.

### 3.3.2. Statistical analysis

statistical study was carried out for the comparison of the results and the implementation evidence of significant differences between the samples for some tests (the phenolic compounds in our case) performed in 3 testing, applying an analysis of variance (ANOVA) followed by the Newman-keuls test at using excel **Annex 7**. The degree of significance of the results is taken at the probability  $p < 0.05$

The enthalpies and entropies of the activation of reactant molecules as a whole complex. According to the activated complex theory, the overall oxidation reaction is preceded by the formation of an activated complex in which reactant molecules rearrange their chemical

structure and bonds in an intimate relation to each other, leading to the intermolecular reactions required for the creation of final products of lipid oxidation.

### 3.3.3. Enthalpy and entropy results

The values of frequency factor and the activation energy  $E_a$  were directly related to the entropies and enthalpies of formation of the activated complex, which was in agreement with the transition- state theory of reaction rates, as shows in the **figures 12, 13, 14**.

#### - *Low phenol content*

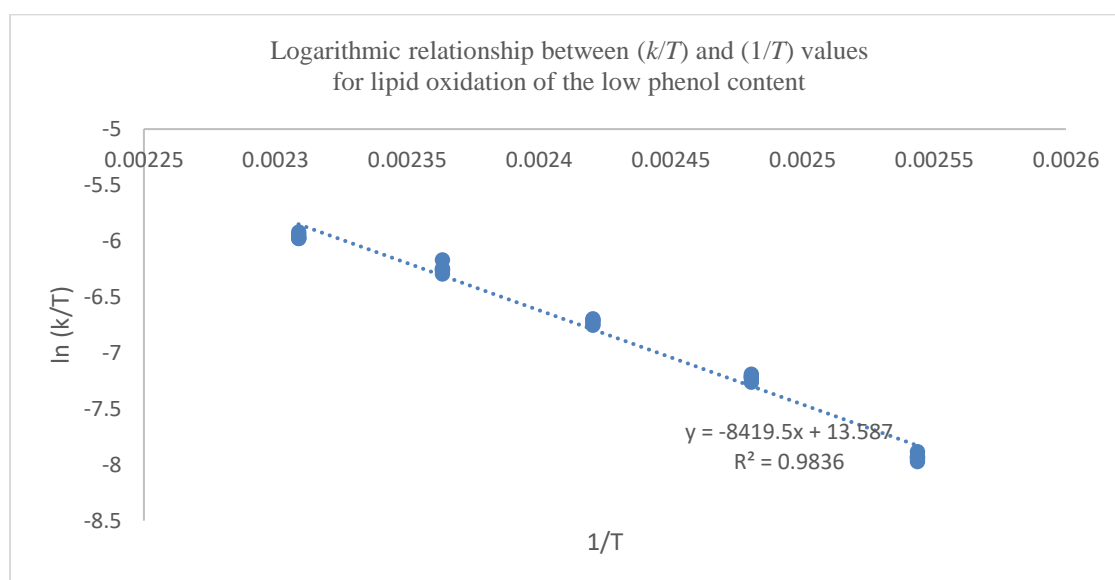


Figure 9. Logarithmic relationship between  $(k/T)$  and  $(1/T)$  values for low phenol content

#### - *Medium phenol content*

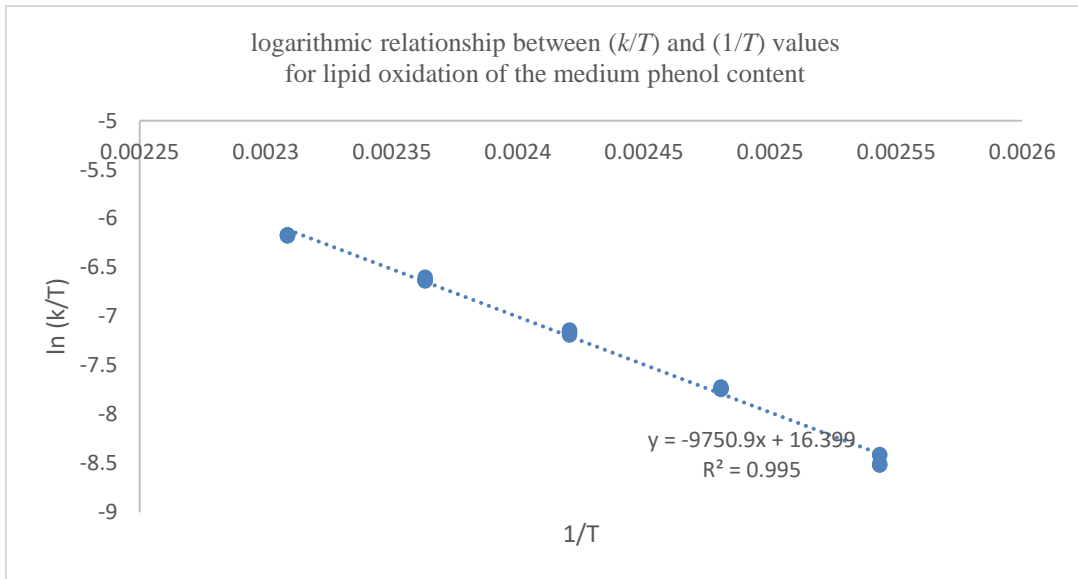


Figure 10. Logarithmic relationship between  $(k/T)$  and  $(1/T)$  values for medium phenol content

- **High phenol content**

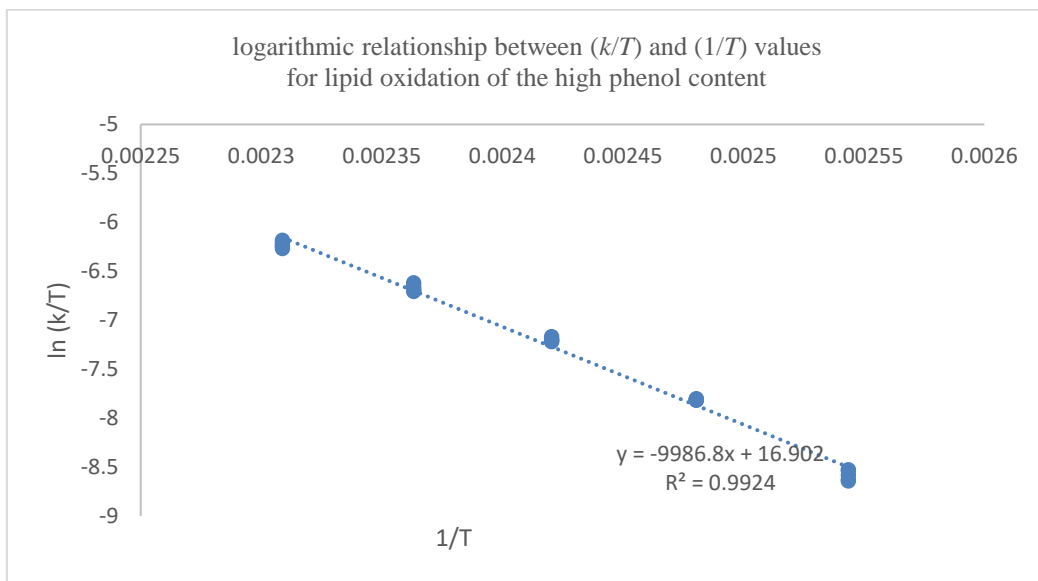


Figure 11. Logarithmic relationship between  $(k/T)$  and  $(1/T)$  values for high phenol content

The obtained results of the enthalpic and entropic parameters are represented in the **Table 8**.

Table 8. Enthalpic and entropic parameters results

	Arbequina cv	Blend olive	Cobrançosa cv
a	-8419	-9750,9	-9986,8
b	13,587	16,399	16,902
$\Delta S^{++}$ (J/(mol K))	-153	-129	-125
$\Delta H^{++}$ (J/mol)	69996	81069	83030

The activated complex in thermodynamic equilibrium with the reactant molecules and has higher energy than both the reactants and final products. The concentration and dissociation rate of the activated complex determine the overall reaction rate of lipid oxidation, which is in turn affected by the enthalpy,  $\Delta H^{++}$ , and entropy,  $\Delta S^{++}$ , of the formation of the activated complex. The values of  $\Delta H^{++}$  and  $\Delta S^{++}$  are considered to be the thermal energy required for the steric changes and level of disorder, respectively, of the activated reactant molecules. The more positive values of  $\Delta H^{++}$ , which reflect the more endothermic nature of the activated complex formation, and the greater negative values of  $\Delta S^{++}$ , which imply that fewer numbers of reactants are involved in the activated complex, show lower reaction rate of lipid oxidation or higher oxidative stability of the corresponding lipid system these results show in particular that the oxidation reaction of olive oil at different concentrations of total phenol content is endothermic since its molar reaction enthalpy  $\Delta H^{++}$  is positive. The negative values  $\Delta S^{++}$  indicate that the activated complexes are more ordered than the molecules of the reactants in the solution, the negative values  $\Delta S^{++}$  indicate fewer numbers of species in the activated complex state.

- *Gibbs free energy*

The table 9 below gives results about Gibbs free energy extract from  $\Delta S^{++}$  and  $\Delta H^{++}$

Table 9 Gibbs results

Free Gibbs energy of activation ( $DG^{++}$ , J/mol)			
Temperature °K	Arbequina olive oil	Blend olive oil	Cobrançosa oil
393.15	<b>130013</b>	<b>131895</b>	<b>132212</b>
403.15	<b>131540</b>	<b>133188</b>	<b>133463</b>
413.15	<b>133066</b>	<b>134481</b>	<b>134714</b>
423.15	<b>134593</b>	<b>135773</b>	<b>135965</b>
433.15	<b>136119</b>	<b>137066</b>	<b>137216</b>

From the Table 9 we can constate when the temperature increases the value of  $\Delta G^{++}$  increase and when the total phenol content increases the value of  $\Delta G^{++}$  increase with it

# **4. Conclusion and perspectives**



Our investigation showed that olive oils that we studied from different varieties and countries were different regarding their chemical parameters such as the peroxide value, acidity, and composition and physical parameters such as the specific coefficients of extinction  $E_{232}$  and  $E_{268}$ .

The measurement of the thermal stability as induction time by Rancimat at different temperatures (120, 130, 140, 150 and 160°C) revealed a strong dependency from the temperature.

Our investigation was close to studies done before which confirm the analyses done and from that we can conclude that the olive oil is endothermic

The Rancimat method was an accurate and effective method to investigate the kinetic data of lipid oxidation in olive oils at elevated temperature such as 160°C. The lowest value for the temperature coefficient was calculated for the low phenol content.

The results of the kinetic calculations were also in good agreement with the studies done before. Thus, the calculation of different kinetic parameters such as activation energy or enthalpies ( $\Delta H$ ) and entropies ( $\Delta S$ ) could be important factors to assess the thermal stability of olive oils. Our kinetic studies demonstrated that the oxidative stability in olive oils is affected by the phenol content;

Due to its relevance it is suggested that in future studies the tocopherol contents as well as the fatty acid composition should be assessed and further used aiming to justify the differences observed at the kinetic-thermodynamic level.

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# 6. Annexes

## Annex 1

### INTERNATIONAL OLEICOL CONCEPT Standards (IOC, 2015)

free acidity

- Extra virgin olive oil:  $\leq 0.8\%$
- Virgin olive oil:  $\leq 2.0\%$
- Common virgin olive oil:  $\leq 3.3\%$
- Lampante virgin olive oil:  $\geq 3.3\%$

Standards (IOC; 2015) peroxide index

- Extra virgin olive oil:  $\leq 20$  meq O<sub>2</sub> / kg
- Virgin olive oil:  $\leq 20$  meq O<sub>2</sub> / kg
- Common virgin olive oil:  $\leq 20$  meq O<sub>2</sub> / kg
- Lampante virgin olive oil: not limited

### Annex 2: free acidity results

Sample	Weight	V. Spend	Acidity (%oleic acid)	Mean	SD	CV	average	Ea
<b>Low phenols</b>	5.0033	0.2	0.11	0.2	0.06	33.35	0.16966	73428.7
	5.0019	0.4	0.23					
	5.0025	0.3	0.17					
<b>Medium phenols</b>	5.0037	0.4	0.23	0.2	0.00	0.02	0.22616	84497.7
	5.0034	0.4	0.23					
	5.0021	0.4	0.23					
<b>High phenols</b>	5.0077	0.4	0.23	0.2	0.03	14.42	0.22611	864590
	5.0014	0.4	0.23					
	5.0028	0.3	0.17					

SAMPLE	(g)	K232	K268	K264	K272	K232	Average	SD	cv	Average
<b>L1</b>	0.605	0.5741	0.2527	0.2696	0.2396	<b>1.90</b>	1.98	0.05	2.45	<b>1.98</b>
<b>L2</b>	0.6042	0.5957	0.2573	0.2736	0.2428	<b>1.97</b>				
<b>L3</b>	0.6013	0.5988	0.2332	0.25	0.2206	<b>1.99</b>				
<b>L4</b>	0.6049	0.608	0.2543	0.2725	0.2408	<b>2.01</b>				
<b>L5</b>	0.6017	0.6075	0.2269	0.2426	0.2153	<b>2.02</b>				
<b>M1</b>	0.6058	0.3617	0.1765	0.2024	0.1617	<b>1.19</b>	1.20	0.04	3.55	<b>1.20</b>
<b>M2</b>	0.6021	0.3422	0.2068	0.2353	0.1901	<b>1.14</b>				
<b>M3</b>	0.6027	0.3572	0.3572	0.2351	0.191	<b>1.19</b>				
<b>M4</b>	0.6045	0.366	0.366	0.2479	0.2042	<b>1.21</b>				
<b>M5</b>	0.6058	0.3798	0.3798	0.2405	0.1954	<b>1.25</b>				
<b>H1</b>	0.6036	0.6807	0.2359	0.2563	0.2238	<b>1.13</b>	1.17	0.08	6.99	<b>1.17</b>
<b>H2</b>	0.6008	0.6334	0.2263	0.2488	0.2137	<b>1.05</b>				
<b>H3</b>	0.6029	0.7383	0.2203	0.2429	0.2073	<b>1.22</b>				
<b>H4</b>	0.6015	0.7574	0.1955	0.2157	0.1838	<b>1.26</b>				
<b>H5</b>	0.6026	0.7224	0.2194	0.2413	0.207	<b>1.20</b>				

Annex 3 specific extinction results E<sub>232</sub>; E<sub>268</sub>

K268	Average	SD	cv	Average
0.2088	0.2029	0.01	5.38	<b>0.193</b>
0.2129				
0.1939				
0.2102				
0.1885				
0.1457	0.2459	0.08	32.73	<b>0.245</b>
0.1716				
0.2955				
0.3021				
0.3146				
0.1963	0.1823	0.01	6.88	<b>0.176</b>
0.1877				
0.1831				
0.1622				
0.1820				
<b>Dilution 1:5</b>				

K264	272	$\Delta K$	Average	SD	cv	Média
0.2228	<b>0.1980</b>	-0.0016	-0.00154	0.00058	-37.86637	<b>-0.0020</b>
0.2264	<b>0.2009</b>	-0.0007				
0.2079	<b>0.1834</b>	-0.0017				
0.2252	<b>0.1990</b>	-0.0019				
0.2016	<b>0.1789</b>	-0.0017				
0.1671	<b>0.1335</b>	-0.0046	0.07183	0.00028	0.38640	<b>0.0594</b>
0.1952	<b>0.1579</b>	-0.0050				
0.1945	<b>0.1585</b>	0.1190				
0.2046	<b>0.1689</b>	0.1153				
0.1992	<b>0.1613</b>	0.1344				
0.2133	<b>0.1854</b>	-0.0030	-0.00380	0.00099	-25.92767	<b>-0.0039</b>
0.2063	<b>0.1778</b>	-0.0044				
0.2019	<b>0.1719</b>	-0.0038				
0.1793	<b>0.1528</b>	-0.0038				
0.2002	<b>0.1718</b>	-0.0039				

Annex 5: Rancimat results

	Sample	Time	Mean	SD	CV	log OS	
<b>120 °C</b>	Low phenols	6.75	7.09	0.22	3.15	1.909543	1.95772
		7.07				1.95586	
		7.36				1.99606	
		7.06				1.954445	
		7.19				1.972691	
	Medium phenols	12.71	12.16	0.66	5.46	2.542389	2.49678
		12.54				2.528924	
		12.67				2.539237	
		11.4				2.433613	
		11.47				2.439735	
	High phenols	14.22	13.60	0.70	5.17	2.654649	2.609294
		14.4				2.667228	
		13.58				2.608598	
		12.95				2.561096	
		12.87				2.554899	
<b>130 °C</b>	Low phenols	3.37	3.39	0.09	2.61	1.214913	1.22115
		3.36				1.211941	
		3.41				1.226712	
		3.29				1.190888	
		3.53				1.261298	
	Medium phenols	5.73	5.66	0.06	1.03	1.745716	1.733381
		5.67				1.735189	
		5.57				1.717395	
		5.65				1.731656	
		5.68				1.736951	
	High phenols	6.16	6.12	0.04	0.64	1.818077	1.811546
		6.15				1.816452	
		6.06				1.80171	
		6.12				1.811562	
		6.11				1.809927	
<b>140 °C</b>	Low phenols	2.07	2.01	0.05	2.36	0.727549	0.697913
		2.05				0.71784	
		1.99				0.688135	
		1.96				0.672944	
		1.98				0.683097	
	Medium phenols	3.09	3.11	0.06	1.90	1.128171	1.133837
		3.12				1.137833	
		3.2				1.163151	
		3.09				1.128171	
		3.04				1.111858	
	High phenols	3.22	3.24	0.07	2.06	1.169381	1.175403
		3.14				1.144223	

		3.31				1.196948	
		3.29				1.190888	
		3.24				1.175573	
<b>150 °C</b>	Low phenols	1.13	1.22	0.06	4.53	0.122218	0.198011
		1.24				0.215111	
		1.22				0.198851	
		1.28				0.24686	
		1.23				0.207014	
	Medium phenols	1.78	1.77	0.03	1.47	0.576613	0.57315
		1.73				0.548121	
		1.8				0.587787	
		1.78				0.576613	
		1.78				0.576613	
	High phenols	1.93	1.86	0.06	3.47	0.65752	0.622238
		1.88				0.631272	
		1.83				0.604316	
		1.91				0.647103	
		1.77				0.57098	
<b>160 °C</b>	Low phenols	0.9	0.89	0.02	2.43	-0.10536	-0.11453
		0.91				-0.09431	
		0.86				-0.15082	
		0.88				-0.12783	
		0.91				-0.09431	
	Medium phenols	1.1	1.10	0.01	0.50	0.09531	0.09893
		1.11				0.10436	
		1.1				0.09531	
		1.11				0.10436	
		1.1				0.09531	
	High phenols	1.18	1.17	0.04	3.27	0.165514	0.158282
		1.22				0.198851	
		1.12				0.113329	
		1.19				0.173953	
		1.15				0.139762	

Annex 6: statistical calcul for Ea:

**Low phenols**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	0.992576
R Square	0.985206
Adjusted R Square	0.984563
Standard Error	0.093709
Observations	25

ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	13.45056	13.45056	1531.704	1.5E-22
Residual	23	0.201973	0.008781		
Total	24	13.65253			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	20.61012	0.547174	37.66649	3.57E-22	19.47821	21.74204	19.47821	21.74204
1/T (Kelvin)	-8831.94	225.6674	-39.137	1.5E-22	-9298.77	-8365.11	-9298.77	-8365.11

**Medium phenols**

SUMMARY OUTPUT

<i>Regression Statistics</i>	
Multiple R	
R Square	
Adjusted R Square	
Standard Error	
Observations	

ANOVA

	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	17.81139	17.81139	5037.388	1.9E-28
Residual	0.081324	0.003536		
Total	17.89272			

	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	0.347207	67.45598	6.09E-28	22.70296	24.13947	22.70296	24.13947
1/T (Kelvin)	143.1964	-70.9746	1.9E-28	-10459.5	-9867.08	-10459.5	-9867.08

### **High phenols**

#### SUMMARY OUTPUT

<i>RegressionStatistics</i>	
Multiple R	0.996527
R Square	0.993065
Adjusted R Square	0.992764
Standard Error	0.075245
Observations	25

#### ANOVA

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	18.64805	18.64805	3293.659	2.45E-26
Residual	23	0.130221	0.005662		
Total	24	18.77827			

	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	23.92488	0.43936	54.45398	8.14E-26	23.01599	24.83376	23.01599	24.83376
1/T (Kelvin)	-10399.3	181.2022	-57.3904	2.45E-26	-10774.1	-10024.4	-10774.1	-10024.4

## Annex 7: statistical analysis

Anova: Single Factor

### SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>	
L	3	220286.3	73428.75	3520121	1876.198
M	3	253493.2	84497.72	1417374	1190.535
H	3	259378.5	86459.5	2269587	1506.515

### ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2.96E+08	2	1.48E+08	61.64161	1E-04	5.143253
Within Groups	14414164	6	2402361			
Total	3.11E+08	8				

t-Test: Two-Sample Assuming Equal Variances

	<i>L</i>	<i>M</i>
Mean	73428.75	84497.72
Variance	3520121	1417374
Observations	3	3
Pooled Variance	2468747	
Hypothesized Mean Difference	0	
df	4	
t Stat	-8.62808	
P(T<=t) one-tail	0.000496	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.000992	
t Critical two-tail	2.776445	

t-Test: Two-Sample Assuming Equal Variances

	<i>L</i>	<i>H</i>
Mean	73428.75	86459.5
Variance	3520121	2269587
Observations	3	3
Pooled Variance	2894854	
Hypothesized Mean Difference	0	
df	4	
t Stat	-9.37997	
P(T<=t) one-tail	0,00036	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.00072	
t Critical two-tail	2.776445	

t-Test: Two-Sample Assuming Equal Variances

	<i>M</i>	<i>H</i>
Mean	84497.72	86459.5
Variance	1417374	2269587
Observations	3	3
Pooled Variance	1843481	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.76961	
P(T<=t) one-tail	0.075755	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.151509	
t Critical two-tail	2.776445	

F-Test Two-Sample for Variances

	<i>L</i>	<i>M</i>
Mean	73428.75	84497.72
Variance	3520121	1417374
Observations	3	3
Df	2	2
F	2.483551	
P(F<=f) one-tail	0.287063	
F Critical one-tail	19	

### F-Test Two-Sample for Variances

	<i>L</i>	<i>H</i>
Mean	73428.75	86459.5
Variance	3520121	2269587
Observations	3	3
df	2	2
F	1.550996	
P(F<=f) one-tail	0.392004	
F Critical one-tail	19	

### F-Test Two-Sample for Variances

	<i>M</i>	<i>H</i>
Mean	84497.72	86459.5
Variance	1417374	2269587
Observations	3	3
df	2	2
F	0.624508	
P(F<=f) one-tail	0.384429	
F Critical one-tail	0052632	

