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Physico-chemical Proprieties of Olive Oil

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Abbreviation lists

Ve: Volume at equilibrium

Ia: Acid index

A% : Acidity

Ip: Peroxide index

I_s: Saponification index

I_i: Iodine index

Ie: Esterification index

I%: Impurities

Ir: Refractive index

C.O.I: International olive oil council

C.A: *Codex Alimentarius*

General introduction

Olive farming has been known since the old times .It has known by its deferent proprieties .this latter make it versatile for example it has been used for lamps also for leather and for therapeutic purpose.

The world production of olive is about 2611000 tons annually. The most producer of virgin olive oil in world is: Spain [1]. Today Algeria produces various types of vegetable oils including olive oil; soya oil... This production yields a lot of profits, which contributes to the recovery of the Algerian economy. Algeria has a climate which helps to plant the olive tree, the thing which makes it among the first countries in olive growing in Mediterranean region. This country is considered one of the countries that is interested in olive cultivation because of the high percentage of its consumption of olive oil as it products annually 35000 tons [2].

The quality of olive oil depends with the quality of ground, the condition of climate, the period of harvesting and the maturity of fruits also the method of extraction. We can obtain the olive oil by three methods: either the extraction by cold pressure which gives three matters: oil, margin, grignon, the oil obtained has a poor quality and the wield is so less not exceed the 20 %, this oil called extra virgin olive oil it is a pure oil , or by the second method which called the two- phases centrifugation extraction ,this method gives two parts: the oil and the grignon. The oil obtained is more resistant to oxidation because of the rate of polyphenols degraded is low and the third process is the three- phases centrifugation extraction which gives three matters: oil, grignon, margin this process must be realize under the action of water, the oil obtained is less resistant to oxidation because the rate of polyphenols degraded is so high [3].

Tlemcen is a city which known by its virgin olive oil and each region has a different olive oil because each oil known by its propriety For that we did a characteristic study about the olive oil of Tlemcen and we did its analysis for comparing with norms, we did the analyze for the olive oil of Sabra, Ouzidene, Saf-Saf, the millennial plant, also the commercial olive oil.

Our purpose is to analyze the olive oil of the millennially plant. We have been chose this plant because it has different properties, special fruits also it is so old and it is never ever studied before that, the thing which makes us excited about it.

Chapter I: THEORETICAL PART

I. Production of olive oil

I.1 Production of vegetable oil in Algeria

The Maghreb countries are the most consumer countries of vegetable oils in world and this consumption increases year after year, but with the most demand does not achieve self-sufficiency. For example, Algeria which is the most producer of soya and palm, as it Produces 27% of soya and the same percentage of Palm each year, also it produces sunflower oil but just 9% each year. The production of these oils contributes to the property of Algeria economy and achievement of self- sufficiency in particular [2].

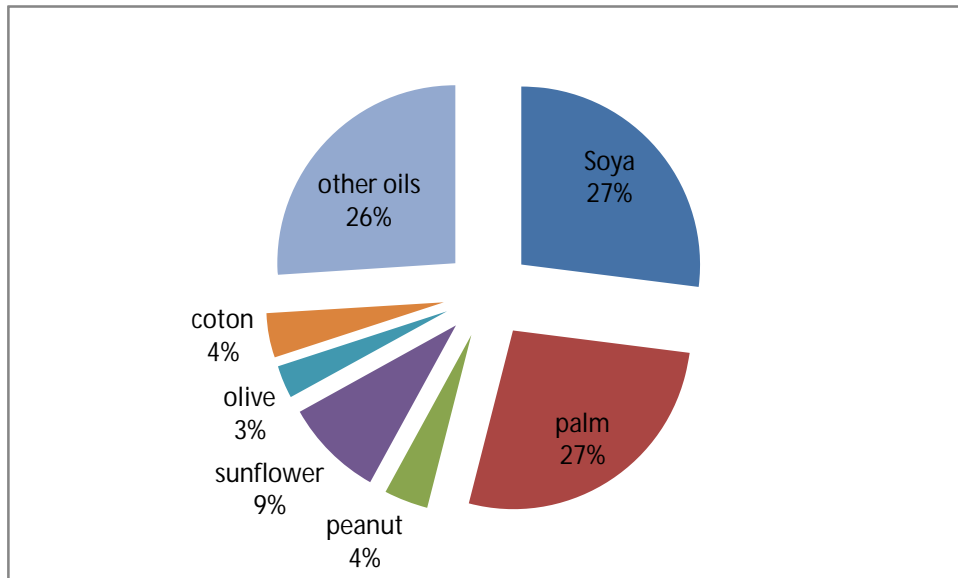


Figure 1: Production of vegetable oils in Algeria “2015 “[2].

I.2. World production of olive oils:

From the year 1990 olive oil production becomes constant between 2,5 to 3,2 million tons annually. This production varies every year because of the change in climatic conditions, of all Spain and Italy are considered as the first in the production of olive oil, it represented 2/3 of the world production of this oil. Generally the countries overlooking the Mediterranean sea are among the most producer countries of olive oil in world and at the same time they are the most consumers of it, for example Spain, Italy, Greece, Turkey. In 2001, these countries produced 95% of the world’s total olive oil [4].

I.3. Algeria olive oil production

Algeria ranks the 8th in the world ranking of olive oil producer countries and the 3rd in the Maghreb region after Tunisia and Morocco. Currently, Algeria consumes 35 million L of olive oil per year and produces 66 mille per year [2].

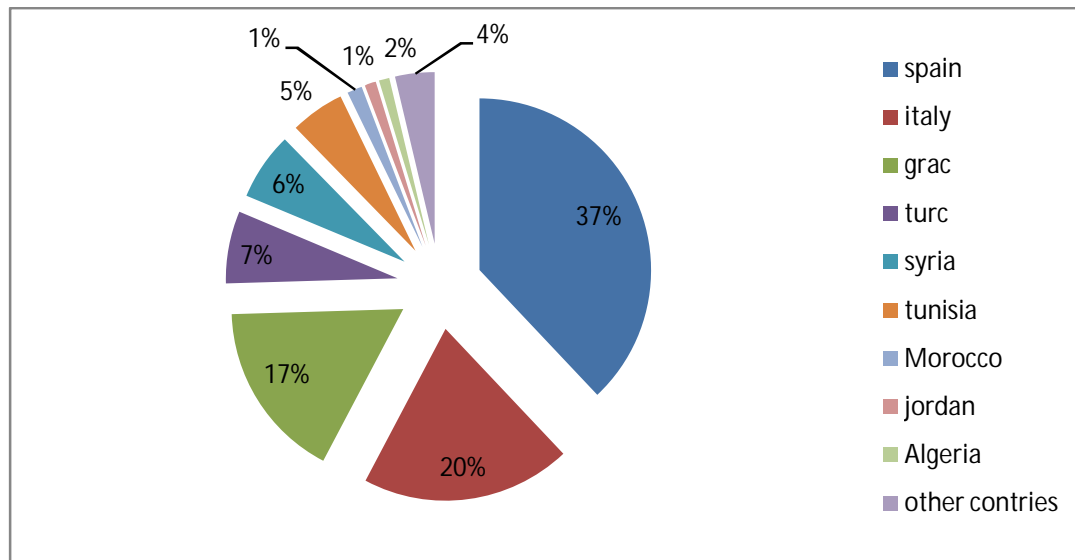


Figure 2: Producer countries of olive oil in 2015 [2].

II. Olive tree

II.1. Story of olive tree

The olive tree is a plant which existed since 5500 years ago, at that time the Greeks and Romans were the number one in field of olive oil so they worked to develop this corp. At the XIXth century, the planting of olive trees arrived in China, Japan, South Africa also Australia, Mediterranean basin. Today the Mediterranean pond is the big olive producer with a percentage of 95% [5].

II.2. Millennial plant

Millennial plant is called “chajara mobaraka”. It has been given this name, because it is an old plant and its known that it is mentioned in Koran, it is among of old olives in Algeria, it is located in an area called Zarifet, it is not far from the city of Tlemcen. Despite the years that have passed but this plant still remains gives beautiful fruits so, it’s a big heritage. It’s a special plant that gives different fruits with specific characters either the quality or the complies with standards [6].



Figure 3: Millennial plant [6].



Figure 4 : Millennial tree seeds [6].

II.3. Virgin olive oil

Virgin olive oil is a pure olive oil that is obtained by mechanical process with no chemical treatment, the used part is the fruits, its kinds are three, the virgin oil which has a good quality but the possibility to find the defects exist, the extra virgin oil which has a super quality so flawless and the current virgin oil, and the only difference between them is the percentage of acidity.

- In the virgin oil the percentage of oleic acid must not be exceed the 2% this percentage represented the free acidity.
- The extra virgin oil has an acidity inferior of 0,8%.
- The current virgin oil which has the higher degree of acidity which is about 3,3% [7].

III. Chemical composition

Each virgin olive oil has two parts:

- The saponifiable function: it is the conversion of an ester to an alcohol or acid under the action of water or a base like triglycerides, phospholipides.
- The unsaponifiable function: it's a part that is insoluble in water but soluble in organic solvents like the sterol, triterpenic alcohol.

According to the international standards this is the most compounds that can find in olive oil and their percentage [8].

Table 1: Olive oil chemical compounds [3].

Compounds	Content
Fatty acid	
Myristic acid	0,05 %
Palmitic acid	7,5 – 20%
Palmitoleic acid	0,3 -3,5%
Heptadecanoic acid	0,3%
Linoleic acid	55: 0 – 83,0%
Oleic acid	0,5 -5,0%
trans fatty acid	
- C18 :1 T	0,05%
- C18 :2 T + C18 :3 T	0,05%
Sterols and triterpenic dialcohol	
➤ Desmethyl sterols	
• Cholesterol	0,5%
• Brassicasterol	0,1%
• Campesterol	4,0 %
➤ Total sterols	1000 mg/Kg
➤ Erythritol et Uvaol	4,5%
Wax C40+C42+C44+C46	250 mg/kg
Stigmastadienes	0,5 mg / kg
Saturated fatty acid in position 2 in triglyceride: Palmitic acid, stearic acid	1,5%
Unsaponifiable	15 g/ kg

IV. Areas studied

Saf-Saf:

It is located in the city of Tlemcen bordered in the north by the region of Bensekran, and from the south by Ain-fazza also from the east by the city of Hennaya, and from the west by the Tlemcen region. The olive oil that we will examine from this area belongs to the farm of HAMADOUCHE. This farm crushes the olives with two phase's centrifugation extraction [9].

Ouzidene:

It is located in a Tlemcen region called Ouzidane, established on 1972. It can give two kinds of olive oil: extra virgin olive oil and virgin olive oil.

- They use three phases centrifugation as a method of extraction.
- They collect olives from different areas of Tlemcen then squeeze them to extract olive oil [9].

Sabra:

Is located from 25 km east of Tlemcen also 20 km west of Maghnia. This region uses the pressure as a method of extraction. It's known by its high quality of olive oil. The kind of ground used for the olive farming is the argilo-huminal [9].

Commercial oil:

The Kemiche olive grove created in 1922 located 2000 Km east of Algiers, situated in the heart of the village "Ifri Ouzelleguen, historical land blessed by the millennial water of the river" Soummam and the mountains of Djurdjura. It gives a virgin olive oil [10].

V. Extraction methods of virgin olive oil

V.1. Story

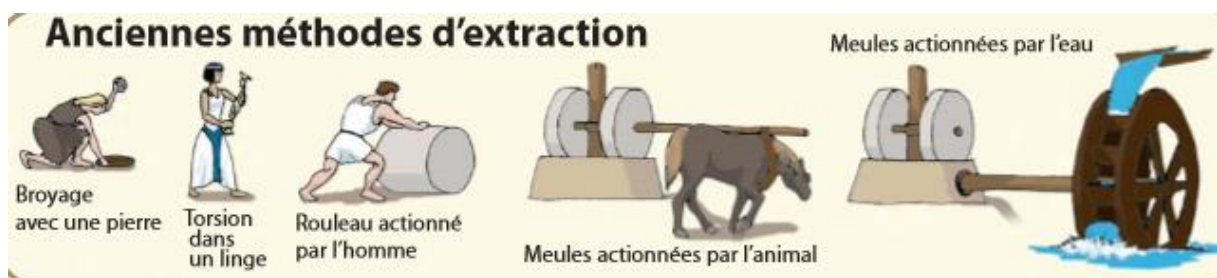


Figure 5: Old methods of olive oil extraction [11].

The extraction of olive oil is known since the beginning and the methods of extraction have been developed over time for improving performance.

At 5000 before B.C, they crushed the vegetal matter by a pestle or a Pierre. After, at 600 before B.C, they used the stone rolls and at 500 before B.C they have been replaced the stone rolls with perpendicular cylindrical grinding wheel operated by hand or by animals. After that at 400 before B.C, they have been disposed the olive in bags and then crushed by a tree trunk, which must be fixed on one side and maneuvered on the other.

The old method of extraction of virgin olive oil is based in the millstone that works by humans or animals, the separation of oil and water is realized by a simple decantation and the liquid must be placed in large boxes of stone after it must be collected by a sheet of metal, in This step they get two parts, the part which floating it is the oil because of its density which is less than water. Today they use another apparatus which called the centrifuge [11].

VI. Technical production

VI.1. Traitement of olives

Either the quality of olive oil or the oil olive production has a relationship with culture. In general, the yield is about 22%. The quality of olive oil is beginning with the moment of plantation of olive, and the duration of storage also the harvest and the conditions of the transport of fruits. The best virgin olive is the oil which has the perfect chemical composition and this latter is related by the equality of acidity and the vitamin content also the relationship between minor compounds [12].

VI.2. Olive harvesting systems

There are several techniques of harvesting of olives which are different from one to another by the nature of soil and the final destination of olives. The harvest of olives should be in the month of October till the month of January. In Algeria, and this period is different from a country to another, for example in France the harvest goes from September to February because climate conditions change, so the speed of maturation of the olive will change also it depends on the variety of olive. This operation can be realized by hand the oil obtained is a table oil and this method is so tedious and takes a lot of time, it is the best operation to get the perfect quality of virgin olive oil because this operation is based on the degree of maturity, another method is the manual comb picking. They use the saplings to bring down the fruits, but this operation can give a worse quality of virgin olive oil because the acidity increases and its taste and aroma change because when the olive falls down causes the suffer damage through soil parasites. This step has a relationship with the maturity degree [3].

The best moment of harvesting is when the fruit of olive gets a dark purple color because the color of olive fruit impacts the olive oil quality and yield, like when the olive fruit is green, the olive is poor in oil. When its color is dark: there is a negative influence on the rates of minor compounds.

The fastest technique of harvesting is the mechanical comb system equipped with an engine, this method saves time but it can cause a little damage for olive trees.

In Spain or Italy the most recent technique is the harvest by vibrating branches, metal clamps encircling the trunk of the olive tree and it applied to the trunk a vibration at a high frequency so the ripe olives fall and distend towards the oil production, this operation is too expensive than the other methods and can cause damages for the young branches of olive trees.

To improve the method of harvesting, it is necessary to install nets under the trees for avoiding the contact directly of the olives with certain metals such as iron or copper also to avoid parasites on the purpose to obtain a virgin olive oil with a good quality because these elements in virgin olive oil should not be exceeded [13].



Figure 6: Harvesting Systems [13].

VI.3. Olive transport

There are several methods of transporting olives, at the time people used utca bags or animals but currently, they transport the olives on crate which has a clear plastic track this allow the circulation of air and for avoiding harmful warning caused by the catabolic activity of the fruits, this method for reducing the danger of crushing because these boxes limit the olive layer. On the other side the transport of olive in dry in jute causes damage drupes which impact on quality of virgin olive oil. To get a good quality of oil it is advisable to grind the olives after a day of harvesting at most [14].

VI.4. Reception and Storage of olive

Before the reception, the lots of olives must be weighed then stored individually according to the degree of maturity; finally these olives must be stored in crates of plastic. Each kind of olive has its method of storage, according to the degree of maturity which gives a specific color to the fruit like:

The black olives must be stored during two to three days; it is the necessary period for the ripening of the fruits this stage requires the savory to do because it is very delicate by cons; the green olive needs just one day to the maximum for the storage to pass directly to the production of olive oil. In general the duration of storage of olive before transformation must not be exceed the three days, because the duration of storage not respecting causes these effects:

- The spontaneous hydrolysis.
- The unfavorable action of enzymatic lipolysis and the harmful effect of the microbial lipolysis produced by the olive micro flora [15].



Figure 7: Storage system “Ouzidene”.
(Picture taken by Ms Zahouane Siham)

VI.5. Olive oil extraction

We distinguish three kinds of olive oil; each kind different to other in system of extraction which makes one of them has its quality, but almost has the same steps, washing, grinding, mixing, and separation of phases [16].

VI.5.1. Washing

Firstly, to clean and eliminate all residues associated with olives fruits and secondly to improve the final appearance of olive oil. The water using in the step of washing must be drinkable [16].



Figure 8: Washing system “Ouzidene”.
(Picture taken by Ms Zahouane Siham)

VI.5.2. Grinding

After storage, the olives must be grinding either by hammer mill or by granite mill; at the end of this operation we obtain a paste. The hammer mill must be adapted to physical condition of the olives and their degree of maturity so the duration of grinding must not be exceed the 20-30 minutes if we do not respect this duration we obtain a poor oil because the polyphenols contain an oil which spontaneously oxidizes in the open air [3].



Figure 9: Grinding system “Ouzidene”.
(Picture taken by Ms Zahouane Siham)

VI.5.3. Mixing

The mixing of olive realized either by a hammer mill or by mill grinding, but the easiest and the fastest method is the hammer mill mixing usually this is achieved to mix the olive to obtain a homogeneous paste. This step must be carried out for a minute to 60 minutes and at temperature above room but not exceeding the 25°C in the case of oil mills with centrifugation equipment [3].



Figure 10: Mixing system “Ouzidene”.
(Picture taken by Ms Zahouane siham)

VI.5.4.Extraction or pressure

The olive oils are different according to the method of obtaining either by pressure which gives two parts the solid past that contains the pulp this part called the grignon and the liquid part which contains a heterogeneous mixture of water and oil or by extraction method, in this operation we use a centrifugal decant which separates the phases deferent of the dough so we get two parts: the first is the olive oil and the second is the mixture of pasty grignon this mixture contains grignon and water. The extraction method are two kinds : the two phases

centrifugation extraction which gives an oil with a high quality because the oil obtained is rich in polyphenols that makes oil resistant to the oxidation but in other side its taste is sour, the other kind called three phases centrifugation extraction it is realized under the action of water it gives three matters : oil, water, grignon we get an oil with a quality less than the oil obtained by two phases centrifugation extraction because its oil is poor in polyphenols [17].

VI.5.5. Conservation of olive oil

- After the separation and obtaining the virgin olive oil, we have to pass to the conservation of this oil. This latter must be put in stainless steel tanks for a complementary decantation. After a month must be transformed in the winery and it will remain protected from the light and the heat until the day of marketing and before this step, the oil must be analyzed and putting in dark bottle before consumption.
- Avoiding the presence of water in oils. It is necessary to put the recipient in temperature of 15°C.
- Avoiding the contact of oil with air [18].

VII. Olive oil analysis

Before the consumption of virgin olive oil, it must be analyzed and compared its values with the values of commercial oil. To classify oil, we must make a physicochemical study to determine its quality. The most olive oil analysis are: refractive index, density, iodine index, esterification index, acid index, peroxide index, saponification index...[19].

Table 2: Olive oil analysis [19].

Parameter	Value
Refractive index	1,466 /1,468
Acid index	4 /6,6
Iodine index	75/95
Saponification index	185 /196
Peroxide index	150/250

Chapter 2: Results and discussion

Part 1: Chemical properties

I. Acid Index

I.1. Definition

It is the mass of potassium hydroxide in milligrams that is necessary to neutralize 1 gram of fatty acid [20].

I.2. Principle

It is the neutralization of free acids by potassium hydroxide solution. The fatty acid reacts with a base like NaOH to give soap according to this reaction:

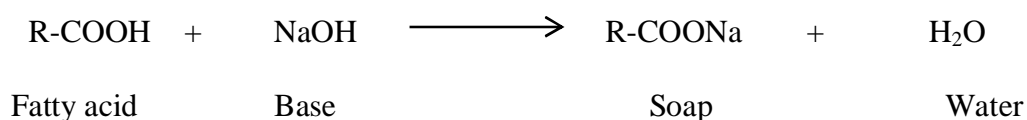
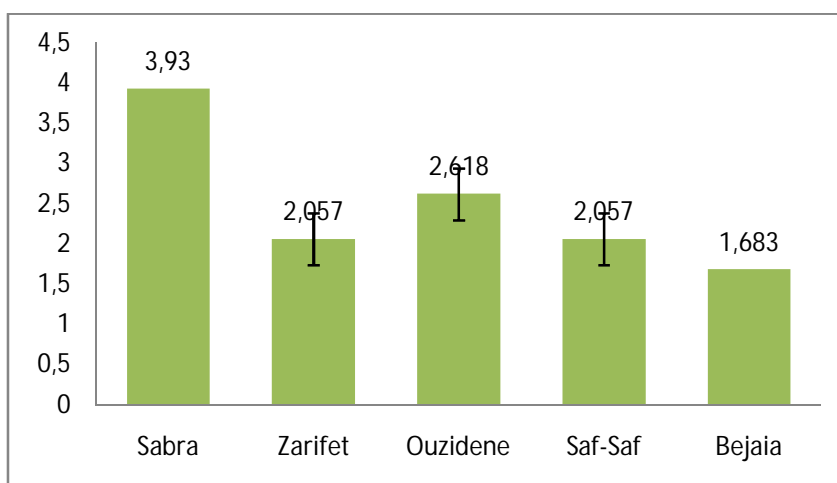


Table 3: Titration results of acid index.

Region	Titrated volume (mL)	Acid index
Sabra	0.7±0.000	3.930±0.000
Zarifet	0.3±0.057	2.057±0.323
Ouzidene	0.4±0.057	2.618±0.323
Saf-Saf	0.3±0.057	2.057±0.323
Bejaia	0.3±0.000	1.683±0.000



Histogram 1: Acid index of olive oils.

The acid index helps us to know the alteration degree by the oil hydrolysis. This index has a relationship with the conservation duration which must not exceed five days, also washing of fruits and the crops duration also the incomplete separation of the deferent phases, all these factors affect in the oil acidity.

The C.O.I norm says that the acid index value must be inferior of 4 mg of NaOH/g oil in the other side, C.A norm says that the same value must be less than 6,6 mg of NaOH/g oil, according to our results we can say that our oils values are in the norms because our olive oils have an acid index less than C.A norm or C.O.I norm, especially our millennial plant oil which has 2,09 as an acid index value, so we can say that our oils have a good conservation [21].

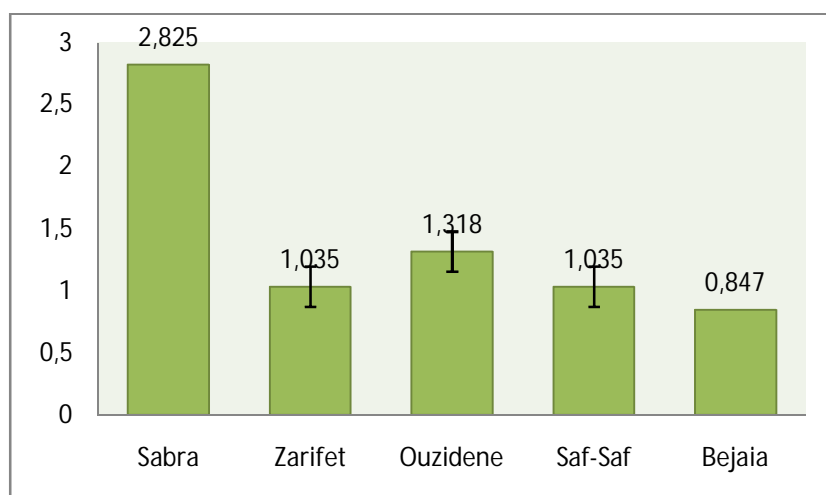
II. Acidity

II.1. Definition

It is the percentage of oleic acid in oil generally; it is a necessary test to know the oil quality [22].

Table 4: Acidity of olive oils.

Region	Acidity %
Sabra	2.825±0.000
Zarifet	1.035±0.163
Ouzidene	1.318±0.162
Saf-Saf	1.035±0.163
Bejaia	0.847±0.000



Histogram 2: Acidity of olive oils.

The C.A norm and the C.O.I norm say that the acidity value must be less than 3,3% and according to our data we can say that our oils are in the norms. All our oils are virgins olive

oils because their acidity are less than 2% only the oil of Sabra which is a current olive oil because its acidity is superior than 2% [3].

III. Peroxide index

III.1. Definition:

It is the number of mille equivalents of active oxygen per kilogram of fatty acid [23].

III.2. Principle

Its principle is to titrate iodine with sodium hydroxide solution (0.01N) released during a reaction, based on the presence of chloroform, KI, acetic acid, starch [23]. Unsaturated fatty acids oxidize in the presence of oxygen to give peroxides, so look at it:

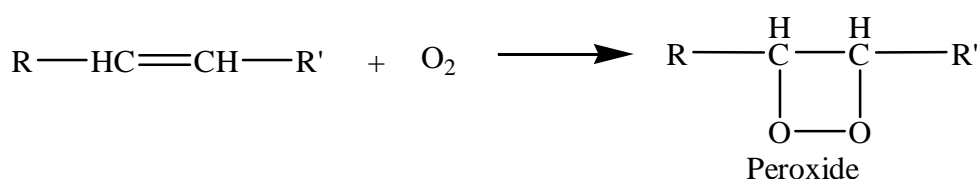
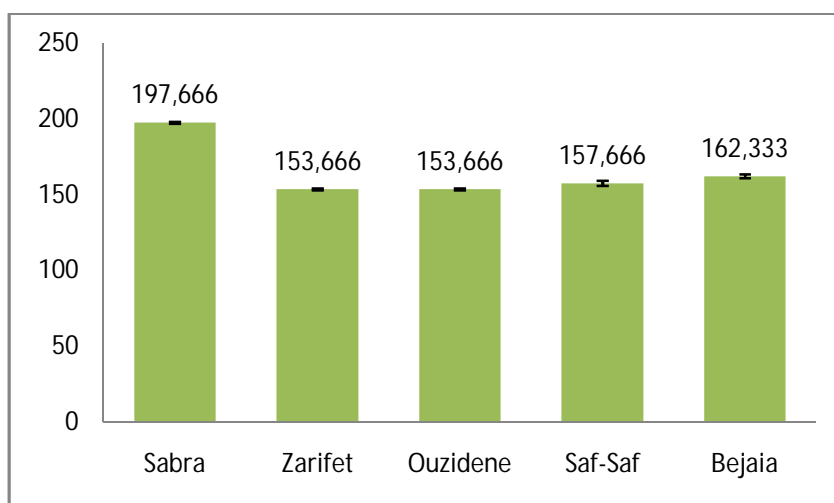


Table 5: Peroxide index of olive oils.

Region	Titrated volume (mL)	Peroxide index
Sabra	20.9±0.057	197.666±0.577
Zarifet	25.3±0.057	153.666±0.577
Ouzidene	21.8±0.115	153.666±0.577
Saf-Saf	21.3±0.152	157.666±1.527
Bejaia	20.9±0.057	162.333±1.154



Histogram 3: Peroxide index of olive oils.

The oxidation of fatty acid in the presence of oxygen gives the peroxide; this one affects in the oil quality especially the organoleptic and chemical proprieties.

The C.A norm says that the peroxide index value must be between 150 mg and 250 mg and according to our results we can say that our olive oils have a peroxide values less than the values mentioned in C.A or C.O.I norms ,so they are in the norms [21].

IV. Saponification index

IV.1. Definition

It is the quantum of potassium hydroxide in milligrams that necessary to saponify 1 gram of fatty acid [24].

IV.2. Principle

The ester reacts with a hot potassium hydroxide to gives the alcohol with the potassium salt this reaction is total and it can gives a born to a new ester according to this reaction [24]:

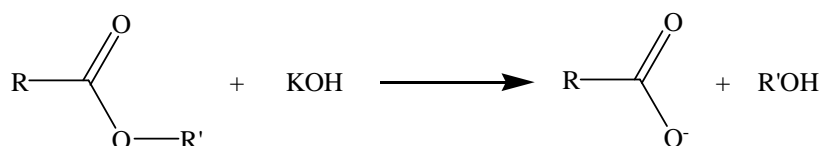
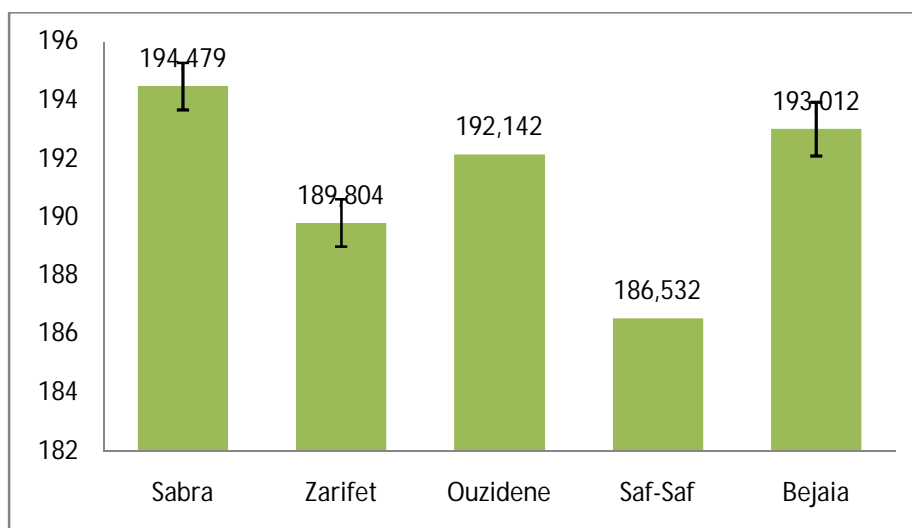


Table 6: Titration results and saponification index.

Region	Titrated volume (mL)	Saponification index
Sabra	1.1±0.057	194.479±0.809
Zarifet	1.4±0.057	189.804±0.810
Ouzidene	1.3±0.000	192.142±0.000
Saf-Saf	1.7±0.000	186.532±0.000
Bejaia	1.1±0.057	193.012±0.922



Histogram 4: Saponification index of olive oils.

The saponification index indicates the length of the fatty chains in oil, plus the saponification index is high plus the oil is rich in short chains of fatty acids. The C.A and the C.O.I norms say that the saponification index value must be between 184 and 196. So according to our results we can say that our oils values are in the norms. So we can conclude that our oils are rich in short chains of fatty acids spatially Sabra oil with the high value of saponification index (194.479 ± 0.809). The saponification index of Zarifet olive oil is (189.804 ± 0.810), the thing which proved that this oil is rich in short chains of fatty acids [22].

V. Iodine index

V.1. Definition

It's the I_2 mass in milligrams that is necessary to fix 100 grams of fatty acids [25].

V.2. Principle

Its principle is based on the fixation of halogen on the ethylic liaison like that [25]:

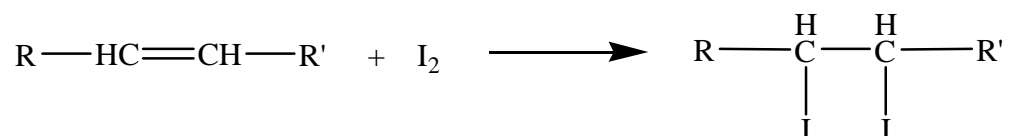
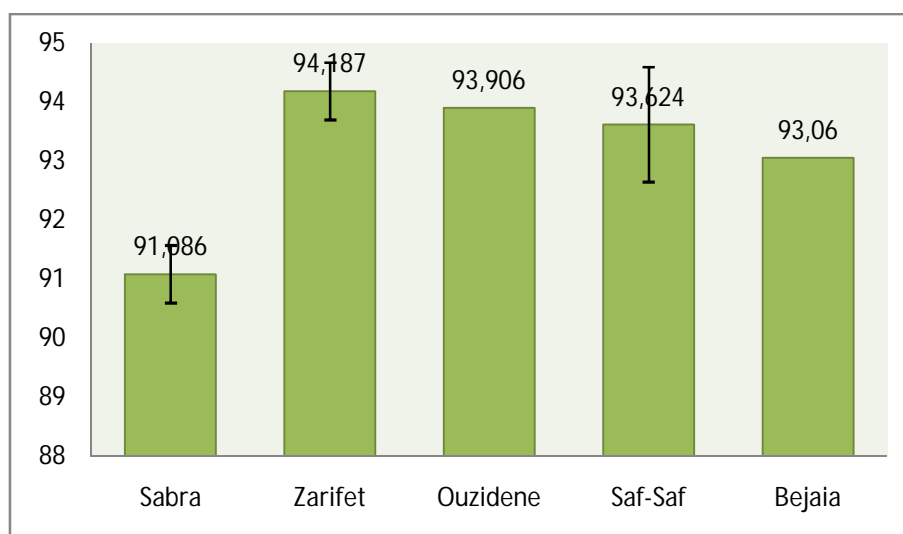


Table 7: Titration results and iodine index.

Region	Titrated volume (mL)	Iodine index
Sabra	12.2±0.057	91.086±0.488
Zarifet	11.8±0.057	94.187±0.487
Auzidene	11.9±0.000	93.906±0.000
Saf-Saf	11.9±0.000	93.624±0.976
Bejaia	12.0±0.000	93.060±0.000



Histogram 5: Iodine index of olive oils.

The iodine index gives the number of insaturations in fatty acid; these insaturations have a relationship with the oxidation degree of oil because if we have oil with a high iodine index we can say that this oil has a high insaturation number.

The C.A norm says that the iodine index must be between 74 and 94. In the other side the C.O.I norm says that this value must be between 74 and 96 so according to our results we can say that our values are in the norms.

We can see that the millennial plant oil has the higher value in the iodine index than the other oils but it stays in the norms. So we can say that our oils have a good quality and they are stables [23].

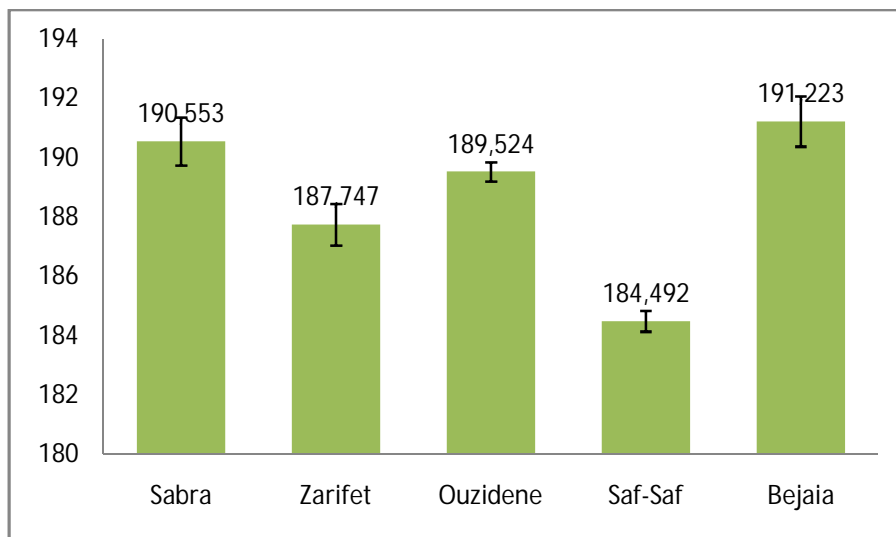
VI. Esterification index

$$I_e = I_s - I_a$$

$$\text{Impurities (\%)} = (I_a / I_s) \times 100$$

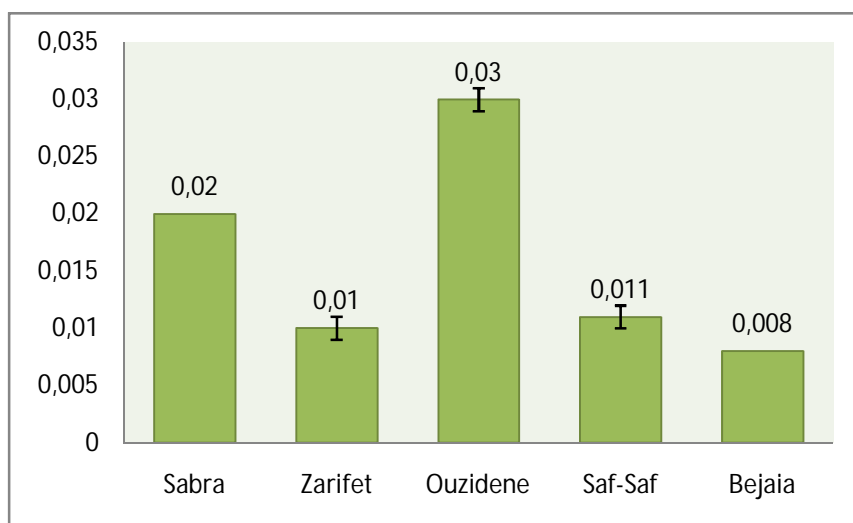
Table 8: Esterification index and impurities.

Region	Esterification index	Impurities
Sabra	190.553±0.803	0.020±0.000
Zarifet	187.747±0.706	0.010±0.030
Ouzidene	189.524±0.323	0.030±0.030
Saf-Saf	184.492±0.353	0.011±0.001
Bejaia	191.223±0.846	0.008±0.000



Histogram 6: Esterification index of olive oils.

We distinguish that our olive oils have a different esterification index.



Histogram 7: % Impurities of olive oils.

According to the national norms ,we can say that each olive oil which has an impurity percentage superior of 1% is out of the norms, and according to our results we can say that only the commercial oil which respect the national norms of impurities because its impurity is less than 1%,the other oils have an impurity superior than 1% because their fatty acids moles numbers are superior than triglycerides moles numbers, and in this case we suppose that the commercial oil has undergone additional treatment than the other oils [22].

Part 2: Physical properties

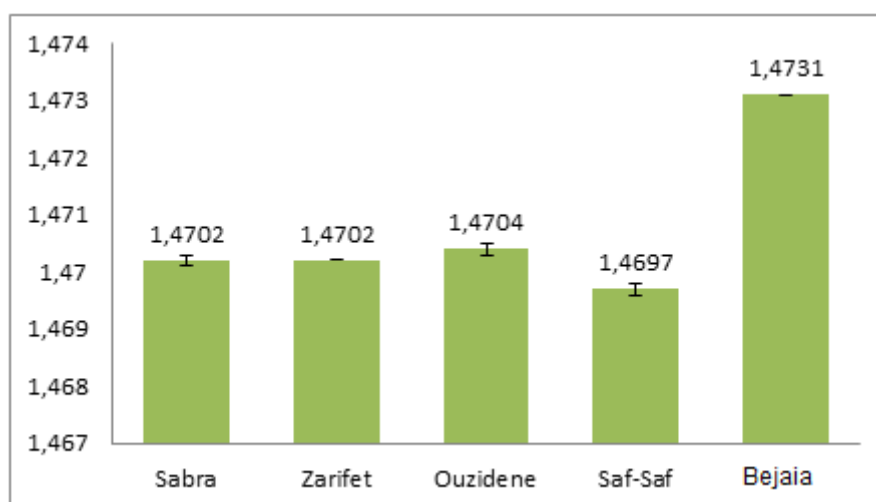
I. Refractive index

I.1. Definition

The refractive index described the comprises of light for explaining the disappearance phenomenon, it is an optic index which hasn't a unite [24].

Table 9: Refractive index olive oils.

Region	Refractive index
Sabra	1.4702±0.0001
Zarifet	1.4702±0.0000
Ouzidene	1.4704±0.0001
Saf-Saf	1.4697±0.0001
Bejaia	1.4731±0.000



Histogram 8: Refractive index of olive oils.

According to the national norms we can say that all our oils have a perfect refractive index because these results are in the norms [25].

II. Color determination

Table 10: Color of olive oils.

Region	Refractive index
Sabra	Dark green
Zarifet	Dark green
Ouzidene	Yellow
Saf-Saf	Yellow
Bejaia	Greenish yellow

We can see that each oil has a different color than the other because our oils are not from the same region and they have not the same method of extraction.

Chapter III: Practical part

Part 1: Chemical properties

I. Acid index

I.1. Operating mode

We weigh 1 g of olive oil then we prepare the ethanoic ether solution, in an Erlenmeyer flask of 200 mL (50/50). After that, we add to each sample 15 mL of this solution and we turn to the assay which realized by a solution of NaOH (0.1 N) already prepared, up to getting a light pink color [21].



Figure 12: Acid index.
(Picture taken by Ms Zahouane Siham)

Law:

$$I_A = V \times 5.611$$

With:

V: NaOH volume titrated (mL);

5.611: Coefficient of acidity.

Table 11: Materials and reagents using in acid index test [22].

Materials	Reagents
Analytical balance	Distilled water
Beaker	Ethano-ether solution (50/50)
Erlenmeyer flask of 250 mL	NaOH solution (0.1 N)
Test tube	
Burette	
Pipette of 15 mL	

II. Acidity

$$A\% = 282.5 \times N \times V \times 100 / P \times 1000$$

With:

282.5: Mass of oleic acid (g /mol);
N: Normality of NaOH (0,1N);
V: Volume of NaOH titrated in mL;
P: Mass of sample in gram.

III. Peroxide index

III.1.Procedure

1 g of oil weighted into Erlenmeyer flask of 250 mL then we add 15mL of acetic acid also 10 mL of chloroform after that we add 1mL of KI . The mixture is stirred for 1 minute. We should cover our solution and put it away from the light for 5 minutes after, we add 75 mL of distilled water, we get a pink solution then we add some drops of starch, the color become purplish bleu. The solution must be titrated with the sodium thiosulfate solution 0.01 N. We complete our dosage until the discoloration of our solution [26].

Table 12: Materials and reagents using in iodine index test [22].

Materials	Reagents
Analytic balance	Distilled water
Erlenmeyer flask	Acetic acid
Test tube of 100 mL	Chloroform
Burette	Saturated Solution of KI
Pipette of :1/10/15 mL	Sodium thiosulfate solution (0.01N)
	Starch

III.2. Preparation of solutions

➤ **Preparation of sodium thiosulfate solution (0.01N)**

We Dissolve 0.395 g of sodium thiosulfate in 500 mL of distilled water.

➤ **Preparation of starch**

We Dissolve 1 g of starch in 100 mL of distilled water.

➤ **Preparation of starch reagent**

We put 1.2 g of I₂ and 2.5 g of KI in 500 mL of distilled water [27].

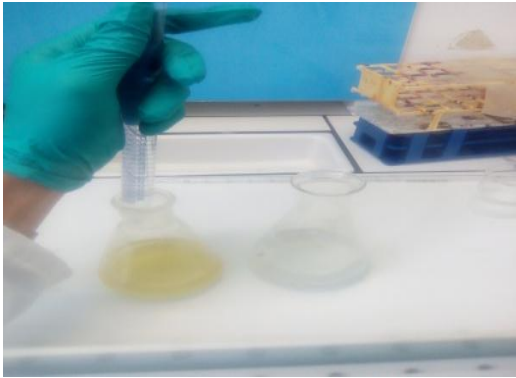


Figure 13: Step 1: The blank test, sample.

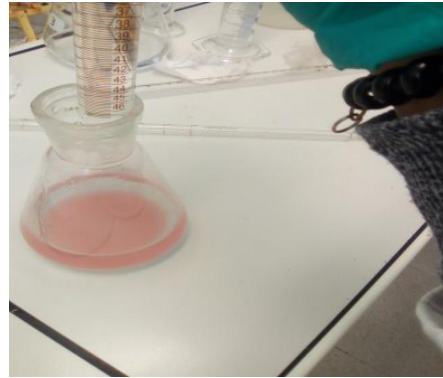


Figure 14: Step 2: After adding water.

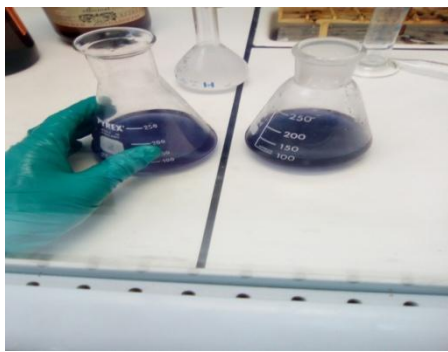


Figure 15: Step 3: After adding starch.

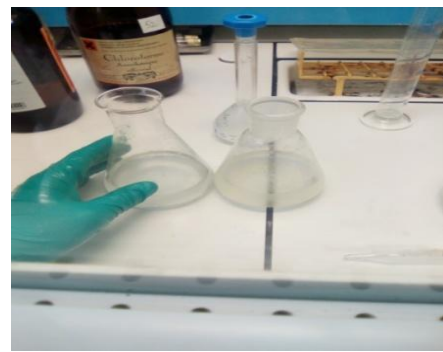


Figure 16: Step 4: After dosage.

(Pictures taken by Ms Zahouane Siham)

Role:

The peroxide index is expressed by this relation:

$$I_p = (V - V_0) \times 10$$

With:

V: Titrated sodium thiosulfate volume (mL);

V₀: Sodium thiosulfate volume of the blank test (mL).

IV. Saponification index

IV.1. Procedure

Before anything, we have to dissolve our oil in an organic solvent, in general the fatty acids dissolve in “ethanol”. So we weight 2 g of fatty acids and we add 25 mL of ethanol, some pumice stones also 20 mL of potassium hydroxide (0.5 N). We heat the mixture for 30 minutes. After that, we add some drops of phenolphthalein. The excess of potassium hydroxide must be treated by hydrochloric acid (0.5 N) until the discoloration of phenolphthalein. The same thing is done for the blank test [28].

Table 13: Materials and reagents using in saponification index test [29].

Materials	Reagents
Beaker	Hydroxide potassium solution (0.5N)
Burette	Hydrochloric acid (0.5 mol/L)
Pipette	Phenolphthalein
Water bath	Ethanol
	Diethyl ether

IV.2. Preparation of solutions

➤ **Preparation of potassium hydroxide solution (0.5 N)**

In flask of 100 mL, we prepare 2.8 g of potassium hydroxide in ethanol.

➤ **Preparation of HCl solution**

We prepare 4.65 g of HCl (33%, d=1.16) in 100 mL of distilled water.

$$I_S = (V - V_E) \times M \times N/p$$

With:

N: Potash normality (0,5N);

P: Test sample (g);

V_E: HCl volume for sample (mL);

V: HCl volume for blank test (mL);

M: Hydroxide potassium molar mass 56.1 g/mol.



Figure17: Solution before saponification index (Picture taken by Ms Zahouane Siham)

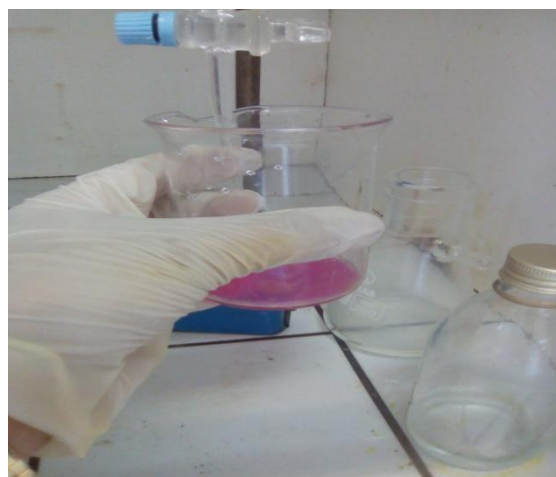


Figure 18: Solution after saponification index (Picture taken by Ms Zahouane Siham)

V. Iodine Index

V.1. Procedure

To realize this operation we use the method of Hubl. We weight 0.15 g of olive oil and we add 30 mL of Hubl reagent made of 15 mL of iodine and the same volume of mercuric chloride. We put the solution far from light while 30 minutes. After that, we add some drops of starch and we dose with sodium thiosulfate solution 0.1 N. We realize also a blank test [30].

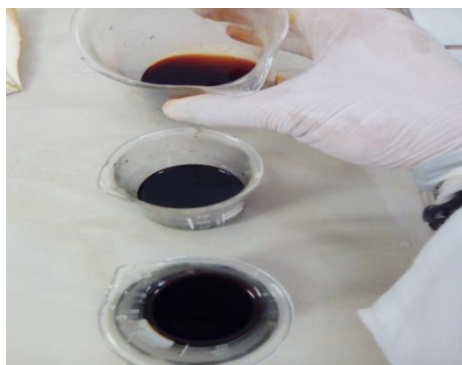


Figure 19: Solution before iodine test.
(Picture taken by Ms Zahouane Siham)



Figure 20: Solution after iodine test.
(Picture taken by Ms Zahouane Siham)

Table 14: Materials and reagents using in iodine index test [30].

Materials	Reagents
Flask of 100 mL	Starch reagent
Beaker	Starch paste
Burette	Mercuric chloride
Analytic balance	Ethanol

IV.2. Preparation of solutions

➤ Hubl reagents

We dissolved 5 g of iodine in 100 g of ethanol 95° and 6 g of mercuric chloride in 100 mL of ethanol 95°. Also, this mix called Hubl reagents, do not forget to filter the mixture and keep it far from light [31].

➤ Sodium thiosulfate solution (0.1N)

We dissolve 0.79 g of sodium thiosulfate in 100 mL of distilled water.

➤ Starch

We dissolve 1 g of starch in 100 mL of distilled hot water.

$$I_T = (V_0 - V) \times 12.69 \times N/P$$

With:

V₀: Volume of titrated thiosulfate solution of blank (mL);

V: Volume of titrated thiosulfate (mL);

N: Normality of thiosulfate solution (0.1N);

P: Test sample (g).

Part 2: Physical properties

I. Refractive index

I.1 Procedure

We do at the first time, the calibration of our apparatus by distilled water; on the screen we can see the refraction index of water: 1.33.

After that we analyze our samples, so we submerge the electrode on the bottle of oil. We adjust the refractometer until we see the line in the center of the circle, than we press the read button for see the value of refraction index of each samples [24].



Figure 21: Refractometer.
(Picture taken by Ms Zahouane Siham)

General Conclusion

According to the convention between our laboratory of natural and bioactive substances and the national park, they asked us to analyze an olive oil of a millennial plant, which never ever studied before that, so we are excited about that because it is an old plant, so we did some analysis to compare this oil with the other oils from Tlemcen and a commercial oil from Bejaia.

The oil that we are studied is from a millennial plant that situated in an area called Zarifet located in the south east of Tlemcen.

Our acid index results says that our olive oils have an acid index in the norms either the C.A norm or C.O.I norm, especially the millennial plant oil, for this we can conclude that the oil of Zarifet has a good conservation.

All our olive oils have an acidity inferior than 2% so we can conclude that all these oils are virgins only the oil of Sabra which is a current virgin olive oil .

The peroxide index test that we did proved that our oils have good organoleptic proprieties especially the oil of Zarifet

All our oils that we studied have a good saponification index which respects the national norms so we can conclude that our oils in general rich in short chains in fatty acids especially the oil of Zarifat.

According to our results that we obtained after the iodine index, we can conclude that the olive oil of the millennial plant has the higher instaurations in fatty acids because it has the higher iodine index.

In the other side we can say that the commercial oil has undergone an additional treatment than the other oils according to its percentage of impurities.

According to our refractive results we can conclude that our oils have a good refractive index.

Each olive oil has a deferent color and this difference is due to the characteristics of each region.

In general the physic chemical analyses that we did in millennial plant oil are good and respect the national norms which proved that our oil has a good quality.

Finally, each olive oil has its composition which gives its quality and this quality depends with the culture, the ground, climatic condition, the method of extraction and the conservation, for this we distinguish deferent kinds of olive oil from a region to another.

References

- [1] P. Vossen, Olive oil: history, production, and characteristics of the world's classic oils. *HortScience* **42**, 1093-1100 (2007).
- [2] J.-L. Benassi, M. Labonne, Perspectives pour les oléagineux dans les pays du Maghreb: Algérie, Maroc et Tunisie 2000-2015. *Oléagineux, Corps gras, Lipides* **11**, 92-96 (2004).
- [3] S. Dauguet, M. Saillard, K. Crépon, Guides de bonnes pratiques d'hygiène dans le secteur des oléagineux. *Oléagineux, Corps gras, Lipides* **20**, 124-130 (2013).
- [4] N. Benyahia, K. Zein, Analyse des problèmes de l'industrie de l'huile d'olive et solutions récemment développées. *Sustainable Business Associates: Lausanne*, 1-8 (2003).
- [5] A. Chohin-Kuper, H. Kemmoun, De la théorie à la pratique: le commerce équitable de l'huile d'olive au Maroc. *Cahiers Agricultures* **19**, 17-22 (11) (2010).
- [6] M.S. Guerriche Tlemcen juillet 2012
- [7] Servi, caractères de l'huile d'olive.
- [8] M.-V. Ruiz-Mendez, M.-C. Dobarganes, Olive oil and olive pomace oil refining. *OCL. Oléagineux, corps gras, lipides* **6**, 56-60 (1999).
- [9] M. A. Sarra, D. de MASTER, Etude des paramètres physico-chimiques et organoleptiques de l'huile d'olives de la variété Siguoise dans la région de Tlemcen.
- [10] S. Angles, Les Appellations d'Origine Protégée (AOP) d'huile d'olive de l'Union européenne. Une analyse comparative (Espagne, France, Grèce, Italie et Portugal). *Méditerranée. Revue géographique des pays méditerranéens/Journal of Mediterranean geography*, 79-84 (2007).
- [11] A. M. Vázquez, Appellations d'origine protégée et innovations: la filière «huile d'olive» à Sierra Mágina (Andalousie). *Cahiers Agricultures* **17**, 542-546 (541) (2008).
- [12] J. De Graaff, L. Eppink, Olive oil production and soil conservation in southern Spain, in relation to EU subsidy policies. *Land use policy* **16**, 259-267 (1999).
- [13] S. Veillet, Université d'Avignon, (2010)
- [14] J.-P. Brun, L'oléiculture antique en Provence. Les huileries du département du Var. *Revue archéologique de Narbonnaise*, 11-301 (1986).
- [15] A.C. Caputo, F. Scacchia, P. M. Pelagagge, Disposal of by-products in olive oil industry: waste-to-energy solutions. *Applied Thermal Engineering* **23**, 197-214 (2003).
- [16] M. Guardia-Rubio, M. Ayora-Cañada, A. Ruiz-Medina, Effect of washing on pesticide residues in olives. *Journal of food science* **72**, (2007).
- [17] G. Montedoro, M. Servili, M. Baldioli, E. Miniati, Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry* **40**, 1571-1576 (1992).
- [18] L. Di Giovacchino, M. Solinas, M. Miccoli, Effect of extraction systems on the quality of virgin olive oil. *Journal of the American Oil Chemists' Society* **71**, 1189-1194 (1994)
- [19] I. Gharbi, M. Issaoui, S. Mehri, M. Hammami, Assurance qualité des huileries tunisiennes. *OCL* **22**, A401 (2015).
- [20] C. Kapseu, Production, analyse et applications des huiles végétales en Afrique. *Oléagineux, Corps gras, Lipides* **16**, 215-229 (2009).
- [21] L. Lagardere, H. Lechat, F. Lacoste, Détermination de l'acidité et de l'indice de peroxyde dans les huiles d'olive vierges et dans les huiles raffinées par spectrométrie proche infrarouge à transformée de Fourier. *Oléagineux, Corps gras, Lipides* **11**, 70-75 (2004).
- [22] S. Selka, A. K. Tchouar, (2015).” Etude de Tlemcen sabra.

- [23] D.S.S. Kpoviessi, G.C. Accrombessi, C. Kossouoh, M.M. Soumanou, M. Moudachirou, Propriétés physico-chimiques et composition de l'huile non conventionnelle de pourghère (*Jatropha curcas*) de différentes régions du Benin. *Comptes Rendus Chimie* **7**, 1007-1012 (2004).
- [24] P. Putzeys, J. Brosteaux, L'indice de refraction des proteides. *Bull. Soc. Chim. Biol* **18**, 1681 (1936)
- [25] D. Ollivier, B. Bruckert, C. Noyer, M. Guère, J. Artaud, Multicriteria analysis for the research of adulteration of virgin olive oil by hazelnut and almond oils. *Annales des Falsifications de l'Expertise Chimique et Toxicologique (France)*, (1999).
- [26] J.A. Solís-Fuentes, G. Camey-Ortíz, M. del Rosario Hernández-Medel, F. Pérez-Mendoza, C. Durán-de-Bazúa, Composition, phase behavior and thermal stability of natural edible fat from rambutan (*Nephelium lappaceum* L.) seed. *Bioresource technology* **101**, 799-803 (2010).
- [27] T. J. Schoch, [2] Preparation of starch and the starch fractions. (1957).
- [28] G. Knothe, R. O. Dunn, Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals. *Journal of the American Oil Chemists' Society* **80**, 1021-1026 (2003).
- [29] G. Knothe, Structure indices in FA chemistry. How relevant is the iodine value, *Journal of the American Oil Chemists' Society* **79**, 847-854 (2002).
- [30] S. Muniategui, P. Paseiro, J. Simal, Measurement of the degree of unsaturation of oils and fats using infra-red spectroscopy and its relationship with iodine value. *Grasas y Aceites* **43**, 1-5 (1992).
- [31] K. Ouali, F. Trea, L. Toumi, A. Bairi, D. Maurel, M.-A. Guellati, L'héspéridine, un antioxydant flavonoïde qui diminue le stress oxydatif et prévient les malformations fœtales au cours du diabète gestationnel expérimental. *Phytothérapie* **5**, 204-209 (2007).

Abstract

Olive oil is the fatty matter that extracted from olives; there are three kinds of olive oil: virgin olive oil, extra virgin olive oil and current virgin olive oil. We can distinguish between them when we know the composition of each oil and to know it we have to do some physico-chemical analyses which help us to know the quality of each olive oil. We choose Tlemcen as a region to study and we choose especially an olive oil from a millennial plant located in Zarifet and other oils from Sabra, Saf-Saf, Ouzidene and Bejaia and we analyzed them. Finally, according to our results, we find that our oils have good quality and they respect the norms especially our millennial plant, in which its analysis results are: acid index (2.057 ± 0.323), acidity (1.035 ± 0.163), peroxide index (153.666 ± 0.577), saponification index (189.804 ± 0.810), iodine index (94.187 ± 0.487), esterification index (187.747 ± 0.706), impurities (0.010 ± 0.030), and refractive index (1.4702).

Key words: Virgin olive oil, Physico-chemical analysis, Zarifet, Millennial plant.

Résumé

L'huile d'olive est la matière grasse extraite des olives; il y a trois types d'huile d'olive: huile d'olive vierge, huile d'olive extra vierge et huile d'olive vierge courante. Nous pouvons les distinguer quand nous connaissons la composition de chaque huile, pour ce faire nous devons faire les analyses physico-chimiques, qui nous aident à connaître la qualité de chaque huile d'olive. Nous avons choisi Tlemcen comme région à étudier et nous avons choisi en particulier une huile d'olive d'un arbre millénaire située à Zarifet et d'autres huiles de Tlemcen. En conclusion, nous constatons que nos huiles ont une bonne qualité et qu'elles respectent les normes spécialement notre huile provenant de l'olivier millénaire. Les résultats d'analyse sont: indice d'acide (2.057 ± 0.323), acidité ($1,035 \pm 0,163$), indice de peroxyde ($153,666 \pm 0,577$), indice de saponification ($189,804 \pm 0,810$), indice d'iode ($94,187 \pm 0,487$), indice d'estérification ($187,747 \pm 0,706$), les impuretés ($0,010 \pm 0,030$) et l'indice de réfraction ($1,4702$).

Mots clés: Huile d'olive vierge, Analyse Physico-chimique, Zarifet, Olivier Millénaire.

ملخص
زيت الزيتون هو الدهون المستخرجة من الزيتون. هناك ثلاثة أنواع من زيت الزيتون: زيت الزيتون البكر وزيت الزيتون البكر الممتاز وزيت الزيتون البكر. يمكننا تمييزها عندما نعرف تركيبة كل نطف ، للقيام بذلك ، يجب علينا القيام بتحليلات فيزيائية-كيميائية ، مما يساعدنا على معرفة نوعية كل زيت زيتون. اخترنا تلمسان كمنطقة للدراسة واخترنا على وجه الخصوص زيت الزيتون من شجرة عمرها ألف عام موجودة في زاريفت وزيتون تلمسان الأخرى. في الختام ، نجد أن زيتوننا لديها نوعية جيدة وأنها تلبى المعايير خاصة زيت الزيتون لدينا من آلاف السنين. نتائج التحليل هي: القيمة الحمضية (2.057 ± 0.323) والحموضة (1.035 ± 0.163)، البيروكسيد (153.666 ± 0.577)، التصين (189.804 ± 0.810) واليود (94.187 ± 0.487) ، مؤشر الأسترة (187.747 ± 0.706) ، الشوائب (0.010 ± 0.030) ومؤشر الانكسار (1.4702).

الكلمات المفتاحية: زيت الزيتون البكر ، التحليل الفيزيائي الكيميائي ، زارفت ، نبات الألفية.