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Réf :.....

Mémoire de Fin de Cycle
En vue de l'obtention du diplôme
MASTER

Thème

Analyses phytochimiques du romarin vs

Elaboration d'une huile végétale à valeur ajoutée

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For those which gave me everything without anything in return There are no words to describe how much my parent has meant to me throughout all my life.

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No matter what I do or say, I won't be able to thank you properly. Your presence at my side has always been a source of strength to face the different obstacles.

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You have always been by my side to support me. May this work reflect my gratitude and affection

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to everyone who helped me from far or near to cross a horizon in my life

sabrina

Dedicace

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To man, my precious offer from God, who owes my life, my success and all my respect: my dear father.

To the woman who suffered without letting me suffer, who never said no to my demands and who spared no effort to make me happy: my adorable mother.

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To anyone who helped me from far or near to cross a horizon in my life.

Amina

SUMMARY

List of figures
List of tables

I: BIBLIOGRAPHY

II: MATERIALS AND METHODS

III: RESULTS AND DISCUSSION

List of abbreviations:

BHA: butylatedhydroxyanisole

CE: conventional extraction

CM: conventional maceration

CSE: conventional solvents extraction

DM: dry matter

DPPH: 2, 2-Diphenyl-picrylhydrazyl

DW: dry weight

EO: essential oil

Eth: ethanol

EQ: equation

FFA: free fatty acid

GAE: Gallic Acid Equivalent

MAE: Microwave Assisted Extraction

MHD: Microwave hydrodistillation

MC: moisture content

ppm: parts per million

QE : quercetine

R: rosemary

Rpm: round per minute

TBHQ : tertiary butylhydroquinone

TPC: total phenol content

TFC: total flavonoid content

US: Ultrasound

UAE: Ultrasound Assisseted Extraction

We: weight of the extract

WP: weight of the plant

W/W: weight/weight

W/V: weight/volume

List of tables

List of figures

Vegetable oils are one of the main components of the human diet, accounting for up to 25% of the average calorie intake, and can be consumed directly as refined or virgin oils, or through many other products of the food industry, which are obtained from the seeds or fruits of plants (soybean, rapeseed, sunflower, olive, palm, flax, etc.) **(Yara-Varón *et al*. 2017; Wang 2011).**

Lipid oxidation is a major cause of deterioration during the shelf life and heating process of vegetable oils. Oxidation can cause sensory and chemical changes, as well as a reduction in nutritional value. In addition, it leads to the appearance of dimers, polymers and cyclic monomers that can be potentially toxic. In order to delay, reduce or prevent oxidative deterioration, antioxidants are added to foods, the side effects of some synthetic antioxidants used in food processing, such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene, propyl gallate and tertiary butylhydroquinone (TBHQ), have been reveoled toxicological concerns. To overcome this problem, several studies have been conducted to find natural products with antioxidant activity. Plants, including herbs and spices, are known for their many phytochemicals that could be potential sources of natural antioxidants **(Wang, 2016; Casarotti et Jorge, 2014).**

Rosemary (*Rosmarinus officinalis L*) is popular as a natural antioxidant because of its high antioxidant capacity, fat-soluble property and good heat stability, and has been officially adopted in European regulations (Commission Regulation (EU), 2011) **(Erkan *et al*. 2008).**

In the analysis of different studies on secondary plant metabolites, the extraction of plant material is the first very important step; there are different solid material compounds, Soxhlet extraction (SE), percolation and maceration extraction. These techniques have been used for more than a century for the isolation of polyphenols. However, their applications are uneconomical due to the excessive consumption of time, energy and polluting solvents. Currently, several representative technologies such as ultrasonic, microwaves, supercritical fluids and instantaneous controlled decompression have proven their effectiveness; tend to be fast, convenient, economical, sustainable and environmentally friendly in terms of polyphenol extraction efficiency, reproducibility and overall position **(Asadi et Farahmandfar 2020).**

The objective of this research was to study the enrichment of edible oils with polyphenols and essential oils from rosemary leaves and flowers, after their extraction by various conventional and innovative methods (maceration, ultrasound, microwave) different tests have been considered to be carried out: determination of polyphenols and flavonoids; estimation of antioxidants capacities of rosemary extract and processed oil, heating test, rancimat and sensory evaluation.

This work is subdivided into two parts:

1- Bibliography that summarizes an overview of rosemary plant, extraction methods and enrichment of vegetable oils.

2- Experimental study that deals the extraction and quantification of phenolic compounds of the areal part of rosemary (leaves and flowers).

I.1. Overview of rosemary plant

Rosmarinus officinalis L., commonly known as rosemary, is a shrub belonging to the Lamiaceae family, native from the Mediterranean basin and has been cultivated in many other regions **(Fig.01)**. This plant has been widely used in traditional medicine since antiquity, and it has also been used as a food preservative and flavoring agent **(Elyemni *et al*., 2019)**.

a. **Vernacular names** English: Rosemary; French: Romarin; Arabic:Eklil El Djabel; Kabyle**:**Amzir or Aklil **(Goetz et Ghedira, 2012).**

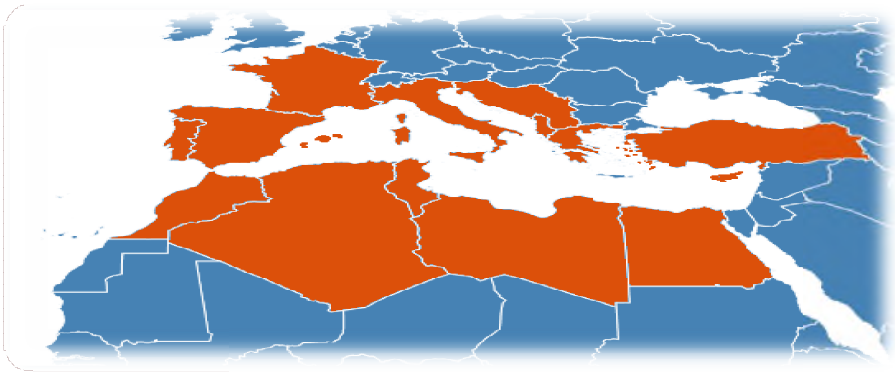


Figure 01: Distribution area of rosemary in the world **(Goetz et Ghedira, 2012).**

I.2. Morphological description

Rosemary is a dense, evergreen, hardy, perennial aromatic herb of 90–200 cm height with small (2–4 cm) pointed sticky and hairy leaves **(Fig.02).** The upper surface of the leaf is dark green whereas it is white below; leaves are resinous. Branches are rigid with fissured bark and stem square, woody and brown. Pale blue small flowers appear in cymose inflorescence **(Shylaja et Peter, 2004**).

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae, labia
Genus: Rosmarinus
Species: *Rosmarinus Officinalis* L.

Figure 02: Rosemary plant **(Gaussen, 1982**).

I.3. Chemical composition of rosemary

A wide variety of useful secondary metabolites has been isolated from *Rosmarinus* plants, including essential oil (EO) and phenolic compounds **(Ribeiro-Santos *et al*., 2015; Sharifi-Rad *et al*., 2020)**. However, the accumulation of bioactive compounds depends on many factors, such as climatic conditions, variety, plant part, extraction technique…etc (**Ribeiro-Santos *et al*., 2015).**This plant includes, in addition to the mineral element (calcium and sodium), lipids, sugar and high levels of vitamins (A, C) **(Tab. I) (Orhan *et al*., 2008; Švarc-Gajić *et al*., 2013).**

Table I: Chemical properties and mineral contents of rosemary (**Švarc-Gajić *et al*., 2013).**

Fraction	Content (100g)	Element	Content (mg/kg)
Total lipids (g)	67.7	Calcium	7792
Sugar(g)	20.7	Magnesium	1635
Fiber(g)	14.1	Phosphorus	1475
Vitamin A (I.U.)	2924	Iron	330
Vitamin C (mg)	21.8	Sodium	2712
Riboflavin(mg)	0.152	Potassium	14916

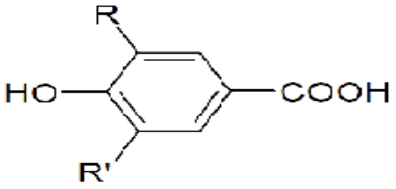
I.3.1. Phenolic composition

Phenolic compounds are a group of aromatic secondary plant metabolites widely spread throughout the plant kingdom. Natural polyphenols range from simple molecules to highly polymerized compounds, the most important are: phenolic acids, flavonoids and tannins **(Lafay et Gil-Izquierdo, 2008).**

a- Phenolic acids

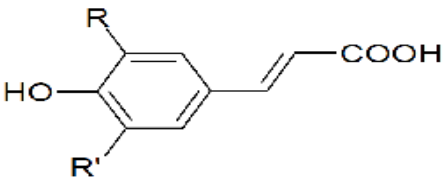
Depending on their structure: derivatives of benzoic acid and derivatives of cinnamic acid. They consist of benzene as basis bond to a carboxylic group (benzoic acids) or to propenoic acid (cinnamic acids **(Fig.03).** Both structures can be found with different hydroxylation levels **(Lafay et Gil-Izquierdo, 2008).**

Benzoic acid derivatives



R = R' = H; *p*-hydroxybenzoic acid
RR = OH, R' = H; protocatechuic acid
R = OCH3, R' = H; vanillic acid
R = R' = OH; gallic acid

Cinnamic acid derivatives



R = R' = H; *p*-coumaric acid
R=OH, R'=H; caffeic acid
R=OCH3, R'=H; ferulic acid
R= R'=OCH3;Sinapic acid

Figure 03: Chemical structures of benzoic acid and cinnamic acid derivatives **(Natella *et al*., 1999).**

The main phenolic acids of rosemary are rosmarinic acid, vanillic acid, caffeic acid, gallic acid, and p-coumaric acid **(Pereira *et al*., 2017).**

b- Flavonoids

Flavonoids are an important class of natural products; particularly, having a polyphenolic structure, widely found in fruits, vegetables and certain beverages **(Panche, 2016).** Flavonoids have a common biosynthetic origin and they all have the same basic skeleton **(Fig.04),** fifteen carbon atoms composed of two aromatic units, C6 cycles (A and B), linked by a C3 chain **(Crozier, 2003).**

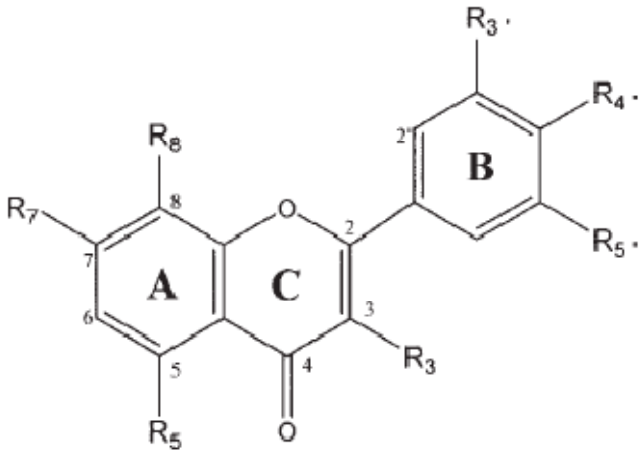


Figure 04: General structure of flavonoid **(Crozier, 2003).**

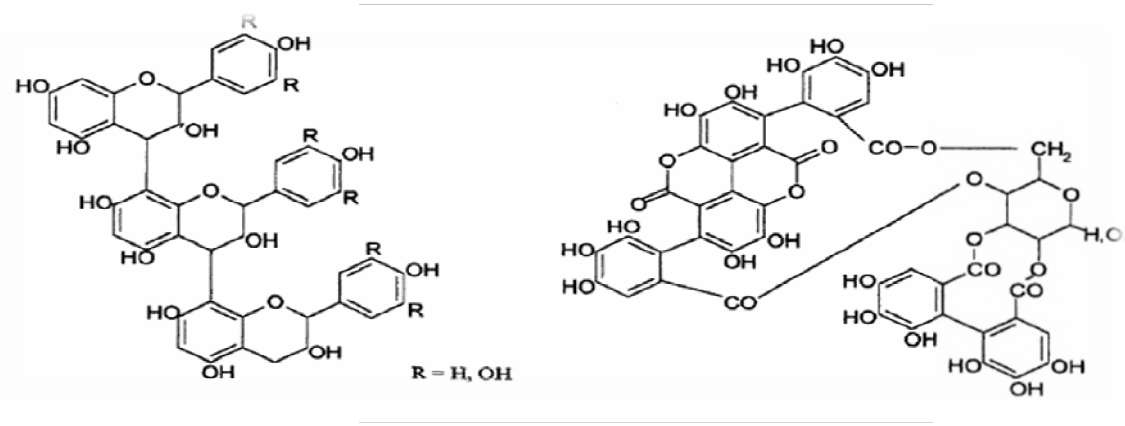
The most common flavonoids in rosemary plant are kaempferol, quercetin and rutin **(Tab.II).**

Table II: Flavonoids of rosemary leaves (**Pereira *et al*., 2017).**

	R3	**R5**	**R7**	**R8**	**R3'**	**R4'**	**R5'**
Kaempferol	OH	OH	OH	H	H	OH	H
Quercetin	OH	OH	OH	H	OH	OH	OH
Rutin	O-$C_{12}H_{21}O_9$	OH	OH	H	OH	OH	H

c - Tannins

Tannins have ability to bound to proteins that form insoluble or soluble tannin-protein complexes.Tannins can be found in nearly all of the legumes, shrubs, vegetables and fruits in the world. According to their chemical structure and properties, tannins are divided into two main groups **(Fig.05)**,hydrolysable **(**carbohydrate ester and phenolic acids) and condensed tannins (dimers, oligomers and/or polymers of flavannes-3-ols or flavannes -3, 4-diols) **(Hassanpour, 2011).**



a- Condensed tannin b- Hydrolysable tannin

Figure 05: Chemical structure of tannins**(McSweeney *et al*.,2001).**

- The rosemary can be considered as one of the tannins-riche plants, they are characterized by a high tannin content condensed **(Abdulhakeem *et al*., 2019).**

I.3.2. Essential oils

Essential oils are volatile substances naturally produced by plants. These are obtained from plant parts like flowers, buds, leaves, seeds, twigs, roots, fruits, bark and wood. Essential oils are stored in cavities, secretory cells, epidemic cells, canals or glandular trichomes **(Valdivieso-Ugrate et el., 2019)**. In general, the constituents of essential oils are terpens

(monoterpenes and sesquerpenes), aromatic compounds(aldehyde, alcohol, phenol, and methoxy derivative), and terpenoids (isoprenoids) **(Tongnuanchan et Benjakul, 2014)**.

a- Terpene hydrocarbons

Terpenes or terpenoids are produced by plants. Plants, especially the flowering ones have a high number of terpenoids with important roles in their biological functions, including in defense, growth, as hormones, pigments, in communication, and as components of electron transfer systems. They originate biosynthetically in the mevalonate pathway from C5 components, known as isoprenoids, sequentially joined as (C5) n structures. Therefore, terpenes are the most common class of chemical compounds found in essential oils **(Fig.06),** they are classified by their number of isoprenoid structures as hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), and tetraterpenes (C40) **(Silva *et al*.,2019).**

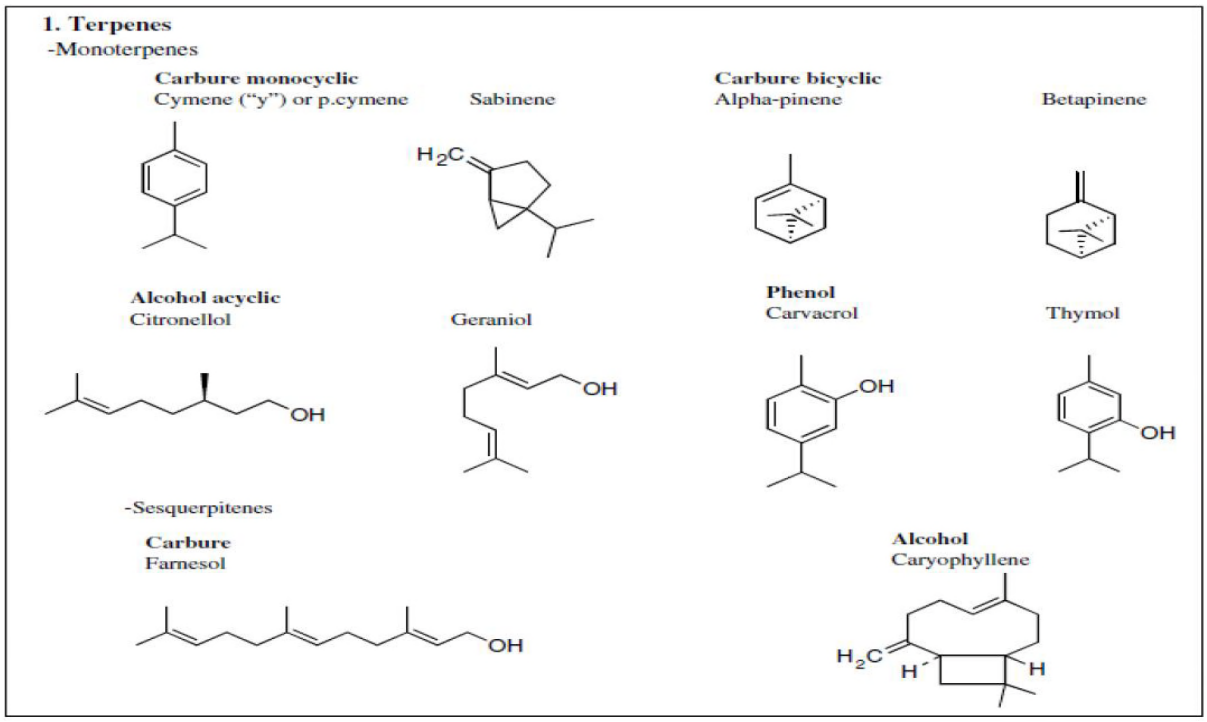


Figure 06 : Chemical structures of terpenes essential oils**(Bakkali *et al*., 2008).**

b- Oxygenated compounds

Oxygenated compounds are important intermediate products in hydrocarbons oxidation **(Zervas, 2005).**These compounds are the combination of C, H, and O, and can be derived from the terpenes, in which they are termed terpenoids**. (Tongnuanchan et Benjakul., 2014).**

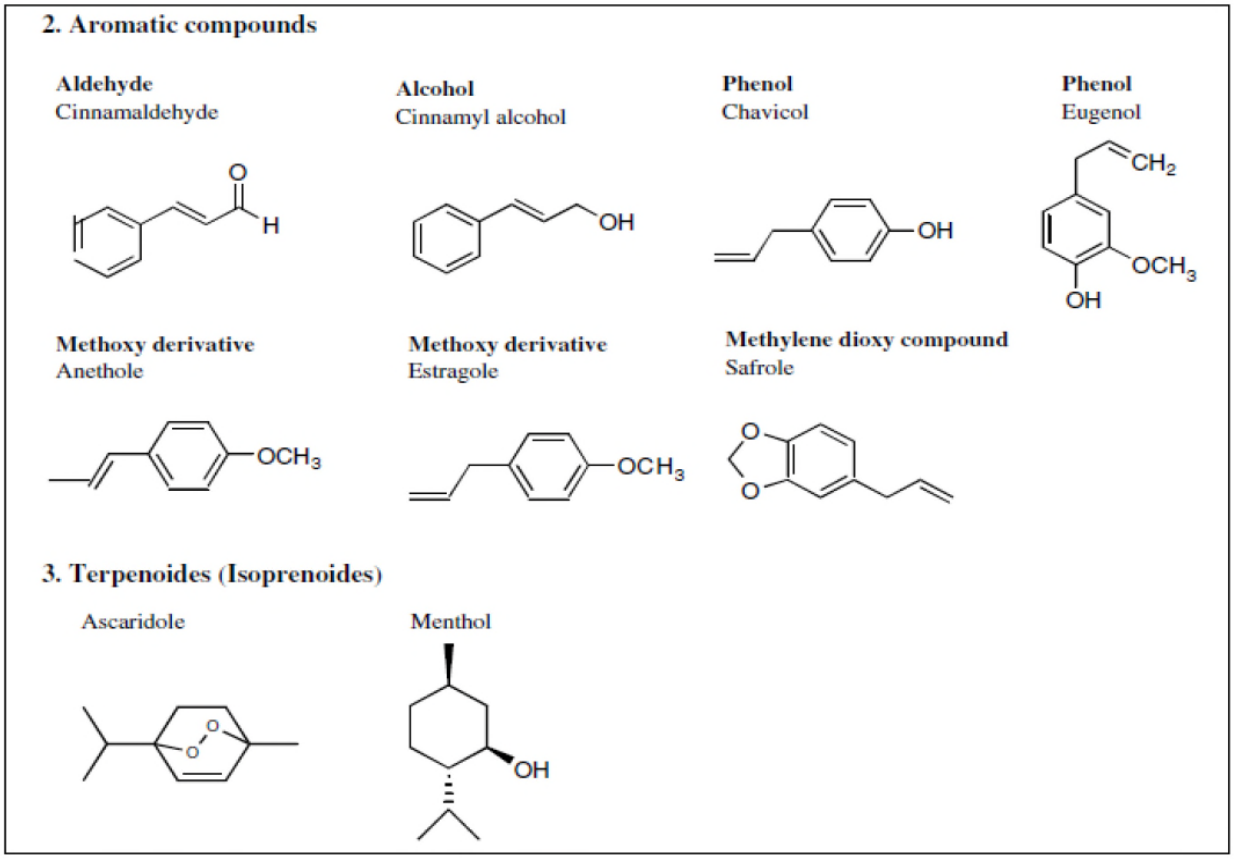


Figure 07: Chemical structures of aromatic components of essential oils **(Bakkali et *al.*, 2008**)

The essential oils of rosemary are main lyphenolic diterpenes such as carnosol, carnosic acid, rosmanol and urosolic acid as shown in **(Fig.08) (Gad,2015).**

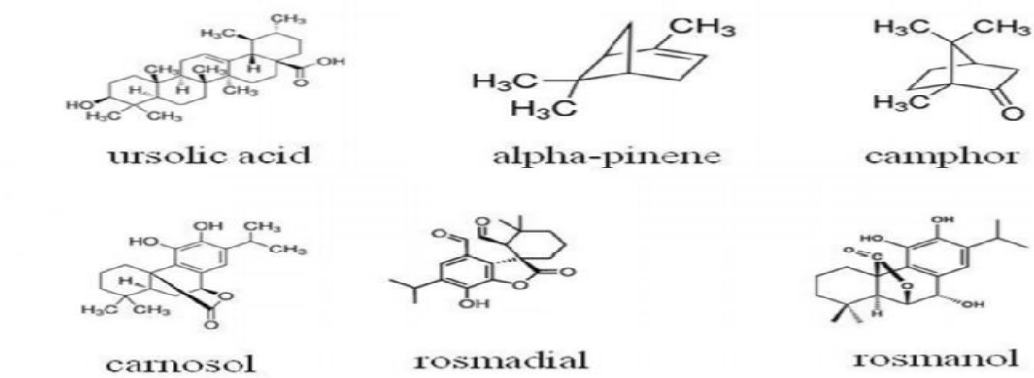


Figure 08: Structures of EO in rosemary extracts **(De Oleveira *et al.*, 2018).**

The chemical composition of the essential oil from rosemary leaves illustrated in table III, obtained by steam distillation and hydro-distillation methods

Table III: Chemical composition of rosemary essential oil **(Oleveira *et al*., 2018).**

Components	Relative contents (%)	
	Steam Distillation	Hydrodistillation
α–Pinene	5.2	0.4
Camphene	3.0	0.3
β–Pinene	5.7	0.3
Myrcene	1.7	tr
p-Cymene	2.2	tr
1,8-Cineol	52.4	31.9
γ–Terpinene	0.5	tr
Sabinene hydrate	0.3	0.4
Terpinolene	0.2	tr
Linalol	1.1	3.9
Camphor	12.6	19.7
Borneol	3.4	12.1
Terpinene-4-ol	0.7	4.0
α–Terpineol	2.1	12.8
Bornyl acetate	1.1	3.1
6 β–Caryophyllene	4.2	3.0

I.3.3. Pharmacological activities

Several preclinical studies reflect the innumerable potentialities of rosemary extracts and its isolated components, especiallydi-terpenes, flavonoids, and phenolic acids. Antioxidantand anti-inflammatory actions of rosmarinic acid, antitumor, hypolipidemic, hypoglycemic, and neuroprotective effects of carnosic acid and carnosol wich is the most prominent and deepened ones. The effects demonstrated by this plant include ability to attenuate asthma and oxidative stress with rosmarinic acid **(Al-Sereiti *et al*., 1999;Oliveira *et al*. 2019; Sharifi-Rad *et al*., 2020).**

a- Antioxidant activity of rosemary

R. officinalis has been used since ancient times where it grows; it is known to be a potent antioxidant and a powerful EO in respect to food preservation, besides to be used for culinary purposes and ornamental plant. In addition to its EO, Antioxidants play a major role in the prevention and treatment of diseases associated with oxidative damage, including cancer, cardiovascular and neurodegenerative diseases **(Aherne *et al*., 2007)**. Reactive oxygen species, including hydrogen peroxide and free radicals, such as superoxide anion (O2 •-) and

hydroxyl radical (HO•), are inevitably produced in living organisms resulting from metabolic processes or from external sources **(Botsoglou *et al*., 2010)**.

Several *in vitro* studies were reviewed regarding the antioxidant activity of the main isolated compounds from rosemary, namely carnosic acid and rosmarinic acid. These bioactive compounds and the essential oil were validated for their antioxidant activity, Also, using the lipid free radicals scavenging activity assays and Rancimat methods (determination of oxidative stability of fat), the bioactive rosemary compounds, have been reported to inhibit lipid per oxidation through the lipid free radical scavenging mechanism **(Andrade *et al*.,2018).**

Extracts prepared from this plant are also widely used in the food industry as a potential anti-oxidant. Indeed, rosemary EO delays oxidation in food-stuff; and therefore, they attract a quiet deal of attention because they can be added to raw or processed foods for preservation purposes, and there by improving the shelf life of some meat products such as sheep and goat cuts, ground meat, and muscle products **(Sharifi-Rad *et al*., 2020).**

Rosemary extract, prepared from the plant with a mixture of ethanol and water (80:20) delayed the oxidation of sunflower oil, therefore increasing its oxidative stability. It especially delayed the formation of primary and secondary oxidation products. Oxidation of the sunflower oil will contribute to alterations in the texture, taste, and odor of foods and also will eliminate the lipid soluble vitamin content of foodstuff; therefore, rosemary can be an important additive in the preservation of sunflower oil along with other vegetable oils that are rich in omega-3 polyunsaturated fatty acids and will contribute to human health **(Sharifi-Rad *et al*., 2020).**

I.4. Extraction methods of phytoconstituents

Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials. Selection of a suitable extraction technique is also important for the standardization of herbal products as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents **(Dhanani *et al*., 2017).**

I.4.1. Conventional extraction technique (CV)

Various extraction techniques most commonly used include conventional techniques such as maceration, percolation, infusion, decoction and steam extraction. There are also

innovative extraction technique such a microwave assisted extraction and ultrasound assisted extraction **(Azmir, Zaidul *et al*., 2013)**.

I.4.2. Innovative extraction techniques

Various novel extraction techniques have been developed for the extraction of bioactive compounds from herbs, including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction **(Dent *et al*., 2015).** Although, these methods have many advantages for the extraction of biomolecules from different plants, mainly concerning extraction time, solvent consumption, extraction yields, and reproducibility **(Oreopoulou *et al*., 2019).**

I.4.3. Microwave assisted extraction

Microwaves are electromagnetic fields in the frequency range from 300MHz to 300GHz. The extraction mechanism of microwave assisted extraction (MAE) involves three sequential steps: first, separation of solutes from the active sites of the solid matrix under increased temperature and pressure, second, diffusion of solvent across the solid matrix; and third release of solutes from the matrix to solvent(Fig.09). To heat up rapidly under microwave radiation, the solvent must have high dielectric constant (which measures the efficiency in which the absorbed microwave energy can be converted into heat inside a material when an electric field is applied) **(Oreopoulou *et al*.,2019).** The extraction efficiency is dependent on the solvent (nature and solvent/sample ration), temperature and pressure, extraction time, radiation power, frequency, sample composition (moisture mainly), and particle size (preferably 0.1 – 2mm) **(Cunha, 2018)**.

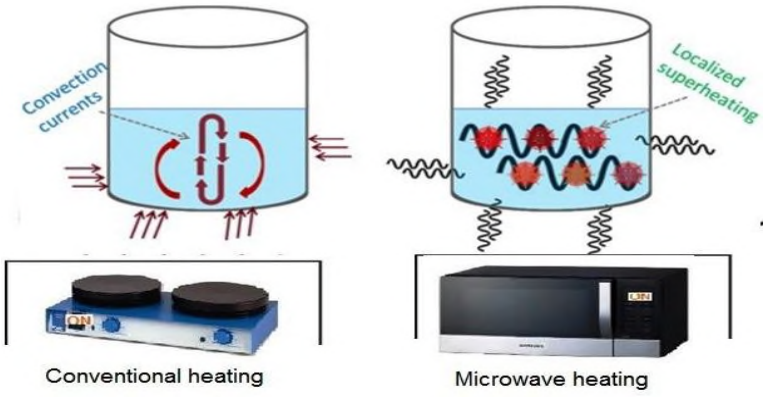


Figure 09: Conventional and microwave heating mechanisms **(Gude *et al*., 2013).**

I.4.4. Ultrasound in extraction processes

Ultrasound assisted extraction in an upcoming extraction technique that can offer high reproducibility in shorter time, higher yields of bioactive compounds, simplified manipulation, decreased temperature during processing, reduced solvent consumption, and lower energy input **(Dent *et al.*, 2015)**. The ultrasonic extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out the cell contents once the walls are broken (Fig.10). The procedure involves the use of ultrasound with frequencies from 20-2000 KHz. Reducing the size of the plant material will increase the number of cells directly exposed to ultrasonically induced cavitation. Ultrasound can facilitate swelling and hydration and so can cause an enlargement in the pores of the cell wall. This will improve the diffusion process and therefore enhance mass transfer. Due to the cavitation, the cells of the plant material are highly disrupted. These small particulates tend to cause problems in solid-liquid separation **(Oreopoulou *et al.*, 2019)**.

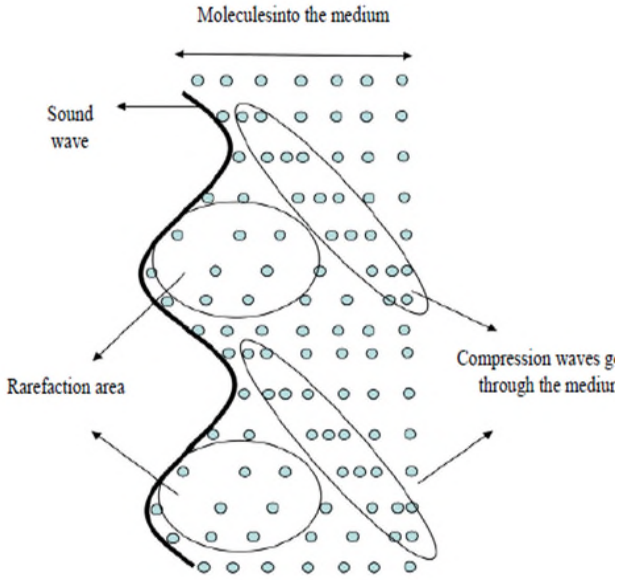


Figure 10: Compression and rarefaction cycles induced by a sound wave **(Achat *et al.*,2012).**

Literature reported the use of the modern technologies MAE and UAE, in the extraction of different active components namely polyphenols and EO from rosemary plant **(Tab IV and V).**

***Table* IV:** Extraction methods of polyphenols from dried rosemary leaves**.**

Methods	Conditions of extraction	References
CM	Water or EtOH / 40°C;	**(Oreopoulou *et al*., 2019).**
Soxhlet	5 g /50 ml ethanol(80%), 30 min, 75°C; 5 g / 150 ml ethanol, hexane,4 h;	**(Juntachote *et al*., 2006).** **(Aziza *et al*., 2008).**
Maceration	50 mg / 2 ml acetone (80%), 45min, 4°C 20 mg / 100 ml distilled water, 24 h, 25°C; 30 mg / 200 methanol (80%), 48 h, 25°C; 1 g / 25 ml methanol (80%), 1 h, 60°C.	**(Saadaoui et *al*., 2006).** **(Milessa *et al*., 2013).** **(Fadili *et al*., 2015).** **(Muhammad *et al*., 2017).**
MAE	800 W, 5 min, ethanol (70%), 5 g / 25 ml; 250 W / 7 min;	**(Švarc *et al*., 2013).** **(Oreopoulou *et al*., 2019).**
UAE	40 KHz, 45 min, 47°C, ethanol; 5 g / 25 ml methanol (70%); 1 g / 20 ml ethanol (90%),30 min, 78°C; 200 W, 1 g / 20 ml ethanol (70%), 12 min, 40°C. 24 KHz / 400 W, 7 min, 40°C;	**(Albu *et al*., 2004).** **(Švarc *et al*., 2013).** **(jacoted-Navarro *et al*., 2015).** **(Hosseini *et al*., 2018).** **(Oreopoulou *et al*., 2019).**

Table V: Extraction method of rosemary essentials oil

	Experimental remarks	Yield Extraction	Solvent	Références
Supercritical fluid extraction	- 64.05 g, SC-CO_2 (0.3 kg/h), 11.5 MPa, 40°C. -Ratio (3.0 g)CO_2 flow rate= 0.3-0.4 ml/min D glass beads = 2 mm	1.03 % (w/w). 0.75(%)	Commercial carbon dioxide (99 % purity) Supercritical fluid	**(Ivanović *et al.*, 2009).** **(Khalili *et al.*, 2017)**
Conventional solvent extraction (CSE)	40 °C,4 h, 50 rpm, 1:6 (w/w)	2.5 ± 0.9 (% w/v) Ethanol 0.605 ± 0.007(% w/v) water	water or Ethanol 96%	**(Rodríguez-Rojo *et al.*, 2012)**
MAE	Method	3.1 ± 1.2 (% w/v) Etanol0.095 ± 0.007 (% w/v)Water	water or Ethanol 96%	**(Rodríguez-Rojo *et al.*, 2012)**
UAE	-Ratio (25g) (1:6 w/w)A Hielscher ultrasonic (400 watts, 24kHz)horn of 22 mm in diameter was used **Discontinuous process, t**= 7 min(30s ON/OFF)**continuous process:** T=40 °Ct= 7 min Membrane 0.45µm. - was extracted 20 g with 200 ml ethanol in an ultrasonic bath (Elma Singen/Htw., type: S 30H, Hohentwiel	**discontinuous process:**2.70 ± 1.5 (% w/v) Etanol0.077 ± 0.004 (% w/v) water **continuous process:**1.10 ± 0.02 (% w/v) Etanol0.075 ± 0.007 (% w/v) water	water or Ethanol 96% Ethanol	**(Rodríguez-Rojo *et al.*, 2012)** **(Choulitoudi *et al.*,**

	Germany) .T= 30 min			**2017)**
Hydrodistillation with a clevenger- type apparatus	-Ratio (20g/200mL) (w/v) t=4hP =atmospheric pressure. -Ratio (200g / 1L) (w/v) ; t= 3 hours - Ratio (100 g/ 1000 ml) t= 3 h -Ratio (100 g / 1l) t= 240 min	23.1 ± 0.86 (mg/g) 0.86 %. 1.15 ± 0.102% (v/w) 1.14%	Water Distilled water distilled water water	**(Liu *et al*., 2011)** ***(Chahboun et al., 2014);(*Hendel *et al., 2016)*** **(Khalili *et al*., 2017)**
Microwave Hydrodistillation (MHD).	Ratio (20g/200mL)(w/v) power = 700 W t=60 min	18.5 ±0.75 (mg/g)	Water	**(Liu *et al*., 2011)**
Hydrodistillation	Ratio (1:10) w/v) ,t= 3.5 h - A yield of 4 mL oil/kg dry herb was obtained.	1.57% 4 ml /kg dry herb	Water Water	**(Marcela *et al*., 2016)** **(Choulitoudi *et al*., 2017)**
Microwave assisted hydro-distillation.	-Ratio(350 g solvent/ 50g sample)	0.026 mL/g	distilled water	**(Karakaya*etal*., 2014)**

(MAHD)	Power=200 W t=75 min; Ratio= (100 g/300 mL) ,t= 30 min. Ratio (50 g/175 ml)room temperature for t=1 h t= 85 min microwave power = 888 w water volume to plant mass ratio of 0.5 ml/g	1.5 mL for 100 g 0.77%	Water distilled water	**(Fazlali *et al.*, 2015)** (**Akhbari *et al.*, 2018)**

I.5. Enrichement of vegetable oils

Deep fat frying is one of the world's oldest and most common food preparation techniques. During the process, heat transfer, cooking of food, water vaporization, crust formation, and several chemical reactions occur constantly. As a result of oil hydrolysis, oxidation, polymerization, Maillard, and other reactions, some decomposition compounds built up in the bulk oil. These materials both reduce quality of the products and create some health problems. Therefore, some precautions are suggested for the continuous and repeated frying operations. To extend the use-life of frying fats and maintain high quality products, oil dilution, frying under modified atmosphere, filtration, adsorbent treatment, and addition of antioxidant additives have been implemented. Thus, addition of natural antioxidant extracts into food lipids to control oxidation in becoming a preferable practice **(Aydeniz *et al*., 2012)**.

Rosemary essential oil is characterized by a widely accepted flavor and suitability to potatoin terms of sensorial properties. Moreover, its antioxidant activities are well known and are due to the synergy between its components. For these reasons, it could represent an interesting product to be used for the impregnation of potatoes, exploiting both its flavor and its stabilizing properties **(Luo *et al*., 2019).**

I.5.1.Vegetable oil of soya

Soybean is the dominant oilseed produced in the world due to its favorable agronomic characteristics, its high-quality protein, and its valuable edible oil. It comprises over a half of all oilseeds produced worldwide. The oil mainly contains neutral lipids, free fatty acids, and polar lipids **(Wang, 2016).**

- **Extraction and composition**

Oil recovered by solvent extraction or mechanical pressing is termed crude soybean oil and it contains various classes of lipids, including neutral lipids (tri-, di-, and mono-acylgl

ycerols), free fatty acids (FFAs), and polar lipids such as phospholipids (PLs). It also contains a minor amount of metals in ppm concentration **(Fig. 11).** When the oil is refined, concentrations of all minor constituents are reduced. The typical composition of crude and refined soybean oil is shown respectively in table VI and figure 12 **(Wang, 2016).**

Table VI: Typical composition of crude and refined vegetable oil **(Wang, 2016).**

Component	Crude oil	Refined oil
Triacylglycerols (%)	**95-97**	**>99**
Phospholipids (%)	**1.5-2.5**	**0.003-0.045**
Unsaponifiable matter (%)	**1.6**	**0.3**
Phytosterols	**0.33**	**0.13**
Tocopherols	**0.15-0.21**	**0.11-0.18**
Hydrocarbons	**0.014**	**0.01**
Free fatty acids (%)	**0.3-0.7**	**<0.05**
Trace metals (ppm)		
Iron	**1-3**	**0.1-0.3**
Copper	**0.03-0.05**	**0.02-0.06**

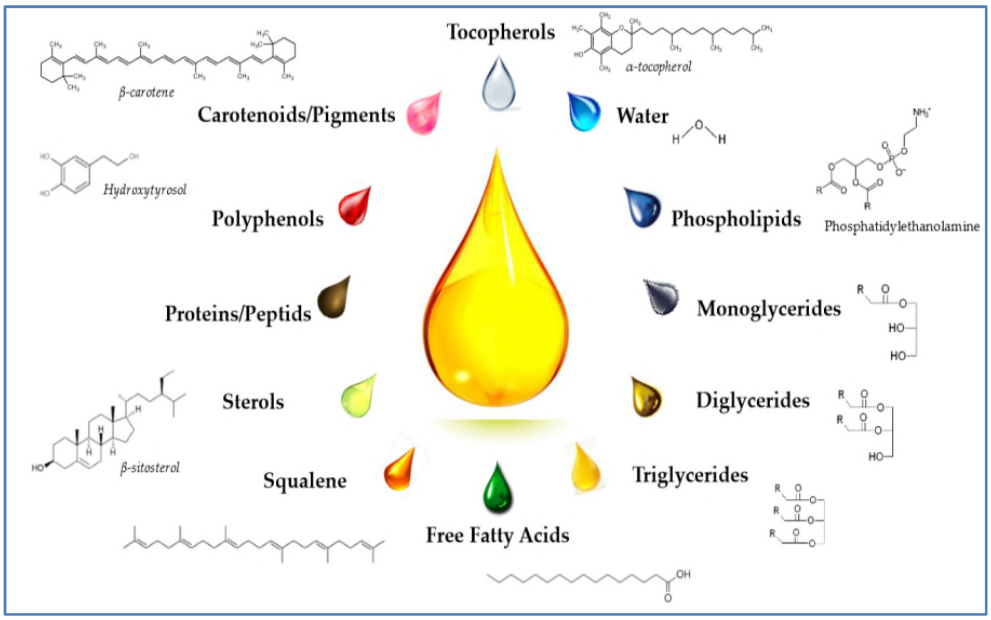


Figure 11: Major and minor components in soya oils **(Yara-Varón *et al.*, 2017).**

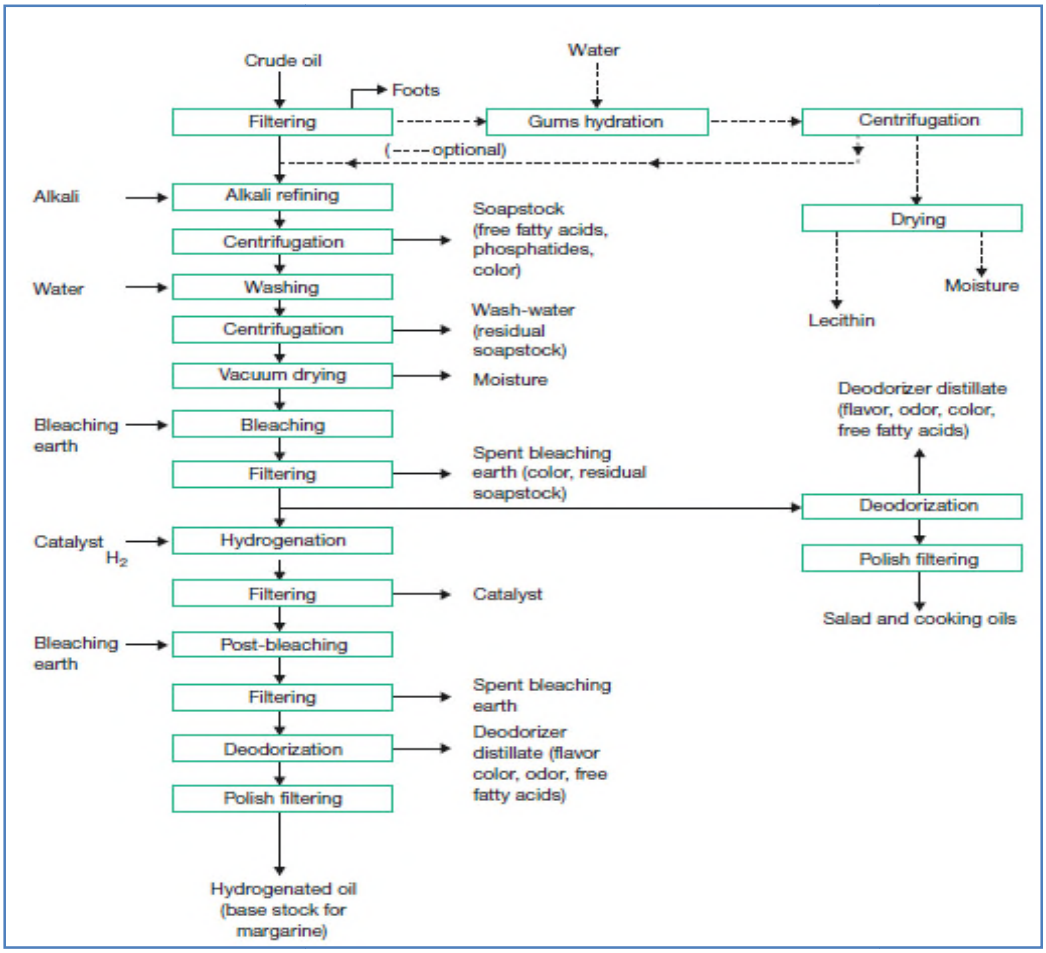


Figure12: Diagram of vegetable oil refining **(Wang, 2016).**

I.5.2. Enrichment methods

a- Extraction solid liquid

A quantity of the vegetable matter (solid) powder is partially dissolved in the oil in this Type of enrichment. Therefore, the movement of the active substances in the oily process is a function of each compound's solubility **(Han *et al.*, 2007).**

b- Liquid-liquid extraction

It consists of making oil in contact with an alcoholic solution of phenols, this is how thes emolecules are transferred to the oily phase as a function of their distribution factor.

The separation of the two phases obtained is performed under vacuum by alcohol elimination and by centrifugation the rest alcohol phase is eliminated **(Han *et al.*, 2007).**

c- Combination of the two methods

In this process, they are only added to the oil after the polyphenols have been removed from the matrix, and the whole is mixed. The separation of the two phases obtained is performed under vacuum by alcohol elimination (**Han *et al*., 2007).**

d- Ultrasound-assisted enrichment

Numerous studies have used edible oil as a solvent to remove interesting substances from various plant matrices **(Achat *et al*., 2012; Li *et al*., 2013; Penalvo *et al*., 2016).** The advantages of ultrasonic sound are linked to the creation of acoustic cavitations, which causes disruption or fragmentation on the surface of the solid matrix. This phenomenon facilitates the acceleration of mass transfer of molecules from solution to adsorbents, resulting in an exponential increase in adsorption rate and shorter equilibrium time. However, the efficiency of the ultrasound is influenced by specific parameters, which include ultrasonic power, sonication time, and temperature. Furthermore, a number of studies reported that prolong high power sonication can have a detrimental effect on the macroporous resins, including structural damages and contamination of the purified extract **(Ismail *et al*., 2020).**

Table VII and VIII present examples of some works of enrich.ent of edible oils with antioxidants

Table VII: Enrichment of refined edible oils with polyphenols.

Matrix	**Experimental Remarks**	**Reference**
Olive leaves	Liquid–liquid enrichment with Microwave phenolic extract (1:1b, 15 min, 600 units/min). Enriched oils obtained a better quality and olive oil was the most enriched.	**(Japón-Luján et Luque de Castro, 2008)**
Olive leaves	Enriched with methanolic extract (500:1b or 250:1b, 20 min shaking,15 min sonication). That enriched oils were better than commercial oils.	**(Chiou *et al.*, 2009)**
Olive fruits, olive fruit particles or olive residue	Oils mixing with olive fruit materials and acids. Preferable conditions are10:1–10:3, 0.5–5 wt.% acid addition in oil-olive fruits mixtures, 90–100°C and 90 min	**(Delaunois *et al.*, 2009)**
Thym flowers	Flavored oil (8:1a, 25 min agitation) showed improved thermal stability than refined corn oil.	(**Karoui *et al.*, 2011)**
Olive pomace	20 g /100 ml of ethanol and the ethanol. In this way, the oil was enriched up to a total phenol concentration of 400µg/ml.	**(Orozco-Solano, *et al.*, 2011)**
Olive pomace	Enriched oil (1:1, 30 min) with total phenols up to 400 g/ml could components of the unsaponifiable fraction so as to improve stability.	**(Aydeniz et Yilmaz, 2012)**
Olive waste	Solid–liquid and liquid–liquid oil enrichments (1:1, 30 min) with dilutions of microwave phenolic extracts. The phenol distribution factor increased with high level of unsaturated fatty acids whereas high saturated fatty-acid content decreased this factor.	**(Rosello-Soto *et al.*, 2015)**
Dried olive leaves	Paradoxical extraction of hydrophilic antioxidants using lipophilic solvents. Virgin oils showed higher extraction efficiency than refined oils(20:3, 2 h, 40°C) as the result of the reverse micelle formation inside.	(**Li *et al.*, 2017)**
Olive leaf	Olive leaf extracts were added to sunflower oil at concentrations of 1,0-1,5 ppm, and mixed vigorously at several conditions (4,000-10,000 rpm and 30-90 sec).	**(Selin Şahin *et al.*, 2017)**

Table VIII: Enrichments of vegetable oils methods with food aroma.

Matrix	Analyte	Operating, condition and remarks	Reference
Air-dried, powdered *Satureja hortens*	Antioxidants	Enriched oils contain 0.1~0.5% of organic solvent extracts, in which *Satureja hortensis L.* ethanol extracts performed the best in oil stabilization	**(Marinova et Yanishlieva, 1997).**
Vanilla pods	Vanillin	US horn, 22.4 KHz 1h pulsed mode (5sec.ON/5sec.OFF).1g vanilla in 100 ml solvent. 140 ppm vanillin concentration in 1h vs 180 ppm in 8h conventional soxhlet.	**(Jadhav *et al.*, 2009)**
Basilic leaves	Essentiel oil (Linalool; Eugénol)	Maceration:150 g/L Linalool = 1.66mg/L ; Eugénol = 0.31mg/L Ultrasound method :150mg/ ⟶ Linalool =3.68mg/L ; Eugénol = 1.34mg/L	**(Jarboui *et al.*, 2010)**
Citrus aurantium peel	Essential oil	Flavoured oil (8:3, 1 h, 20◦C, 100 rpm) showed the highest total volatiles with unchanged fatty acid composition.	**(Karoui *et al.*, 2010)**

Bitter orange peels *(Citrus aurantium L.)*	Monoterpene hydrocarbons (limonee)	500 ml as lipid matrix, orange peels homogenized in the oil. Incubation period (1 h, 2 h and 3 h) and peel quantity (5 g, 10 g and 15 g) were optimized while oil volume (40 ml), incubator temperature (20 ◦C) and shaking speed (100 rpm).	**(Karo *et al.*, 2010).**
Thyme dried flowers (Thymus capitatus)	Essential oils	Antioxidant activities of the thyme-enriched oil were mainly due to the presence of thymol and hydrocarbons such as γ-terpinene and p-cymene.	**(Karoui *et al*., 2016)**

II. Materials and methods

II.1. Choice of matrix

Rosmarinus officinalis, L. originating from the Mediterranean region is an aromatic plant, contain essential oil and polyphenols; Consumers are concerned about the negative effect of synthetic chemicals in food, so it is necessary to find "clean label products". Therefore, there is growing interest in using naturel extracts as alternatives to synthetic additives because of (a) their synergy with other preservation methods, (b) their safety and (c) their specific proprieties as antioxidants, antidiabetics, antimutagens, antitoxicants and antibacterial. In the United States and Europe, rosemary is a unique spice commercially available for use as an antioxidant, rosemary have been used in food preservation, because it prevent oxidation **(Nieto *et al*., 2018)**.Thus, the uses of this plant can lead to the enrichment of oils, which increased its nutritional value **(Jović *et al*., 2018).**

II.2. Chemicals

All solvents and reagents used were of analytical grade. 2, 2-diphenyl-1-picryl-hydrazil (DPPH°), Trichloroacetic acid ($C_2HCl_3O_2$) were purchased from Sigma-Aldrich (Germany). Iron (III) chloride hexahydrate ($Cl_3FeH_{12}O_6$). Potassium Iodure (KI), Methanol, Cyclohexane(C_6H_{12}), Ethanol, potassium ferricyanide ($C_6N_6FeK_3$), Chloroform, potassium per sulfate, ABTS, were supplied from Biochem-chemopharma(UK) however sodium dihydrogenphosphate from VWR (France), Sodium carbonate (Na_2CO_3), Folinciocalteu phenol reagent, aluminum chloride hexahydrate ($AlCl_3H_{12}O_6$). Gallic acid ($C_7H_6O_5$), quercitin, sodium acetate anhydrous ($C_2H_3NaO_2$), , hexane (C_6H_{14}), phenolphthalein, sodium hydroxide (NaOH).

II.3. Plant material

The wild rosemary plant harvest was carried out at the flowering stage; it was collected from Abadou (Bejaia) in 28/02/2020. The geographical position of this region is: 36°38'05.2N and 5°13'46.4S. After identification, the harvested plant materials were washed with running tap water to remove surface contaminants. The samples (leaves and flowers) were dried in a deferent drying oven (nuve 2017, turkey) at 30°C until constant weight, then ground using a grinder (IKA A11 BASIC, Germany) to a granulometry (Sifter,RETCHE) lower than 250µm, prior to extraction after delipidation. Thus, 25g of sieved rosemary was placed in the Soxhlet apparatus cartridge then wet with 50 ml of petroleum ether, then put 200 ml of petroleum ether in the Soxhlet flask heated at 65°C for 2 hours. The step of delipidation aims to eliminate the

lipidic part existing in the plant, after this stage the powder is evaporated in oven at 40°C for 2 hours **(Erkan, Ayranci et Ayranci, 2008).**

II.4.Evaluation of moisture content

Thermal drying method was used in the determination of moisture content of the sample. 5 g of sample were placed in an oven (Binder) to dryness at 103± 2°C, until constant weight. The moisture content (MC) was calculated by the following formula (**Doymaz *et al*., 2004**).

Where W0 correspond to the loss in weight (g) on drying and Wi correspond to the initial weight of sample (g).

$$MC(\%)=(Wi-W0/Wi)\ X\ 100$$

II.5. Extraction procedure

II.5.1. Microwave assisted extraction

A domestic microwave oven (NN-S674MF.Maxipower, China) with cavity dimensions of 22.5 cm × 37.5 cm ×38.6 cm and 2450 kHz working frequency was used. The apparatus was equipped with a digital control system for irradiation time and microwave power (200 to 1000 W) **(Fig.13, 14).** The oven was modified in order to condensate the sample's vapors generated during extraction giving a constant sample volume for the extraction **(Dahmoune *et al*., 2013). Figure 13:** Microwave apparatus

- **Method**

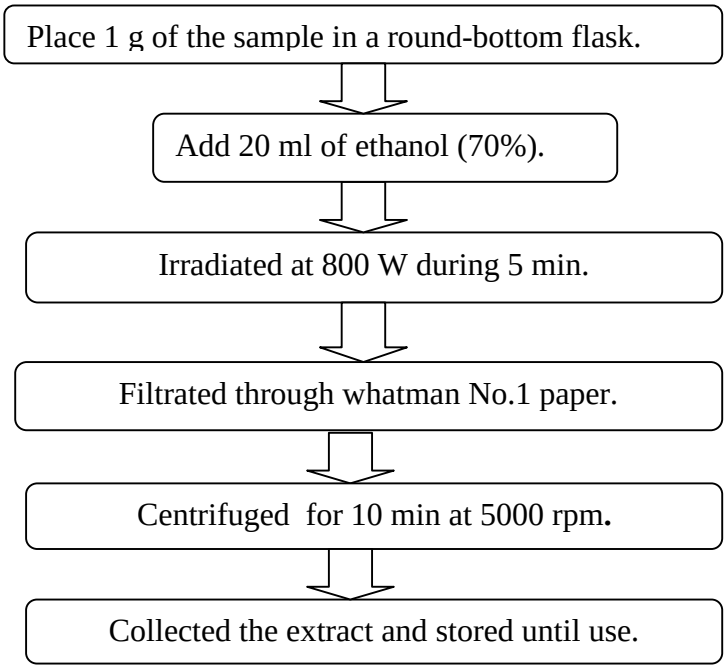


Figure 13: Microwave assisted extraction process **(Dahmoune *et al.*, 2013).**

II.5.2. Ultrasound assisted extraction

The UAE process was carried out using an ultrasound probe at fixed sonication conditions (power of 130 W, a frequency of 20 KHz, and an acoustic energy density (AED) of 35W L-1; amplitude 100%) for 12 min **(Fig.14).**

Figure 14: Ultrasound apparatus.

- **Method**

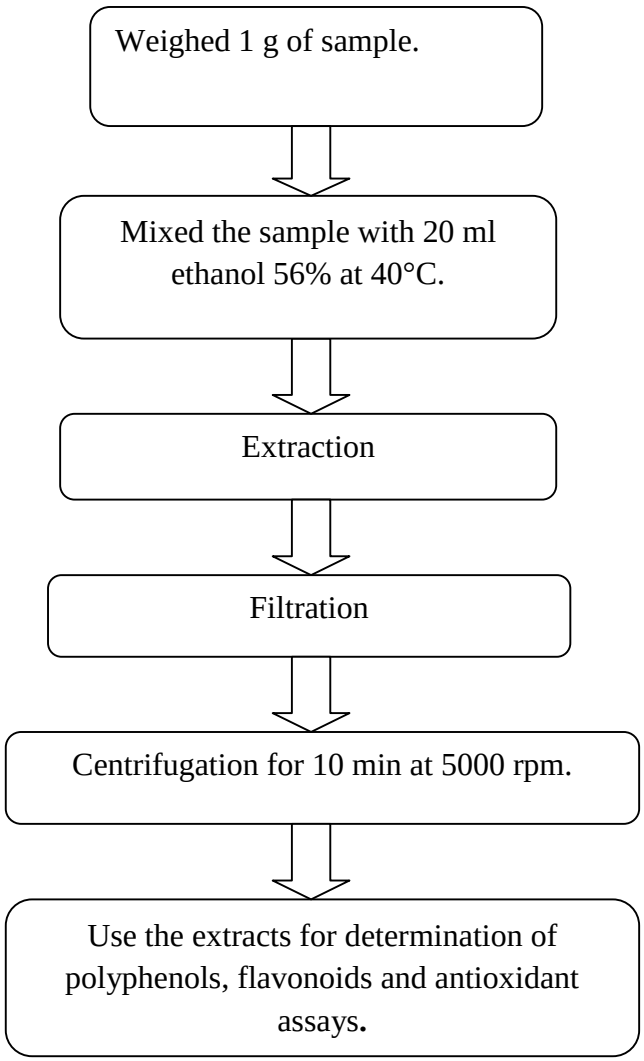


Figure 14: Ultrasound assisted extraction process **(Rodriguez *et al*., 2012).**

II.5.3. Maceration

Maceration is a conventional technique, based on the extracting power of different solvents in use and the application of heat and/or mixing (**Fig.15).** In order to obtain an extract of bioactive compounds from plant **(Azmir *et al*., 2013).**

- **Method**

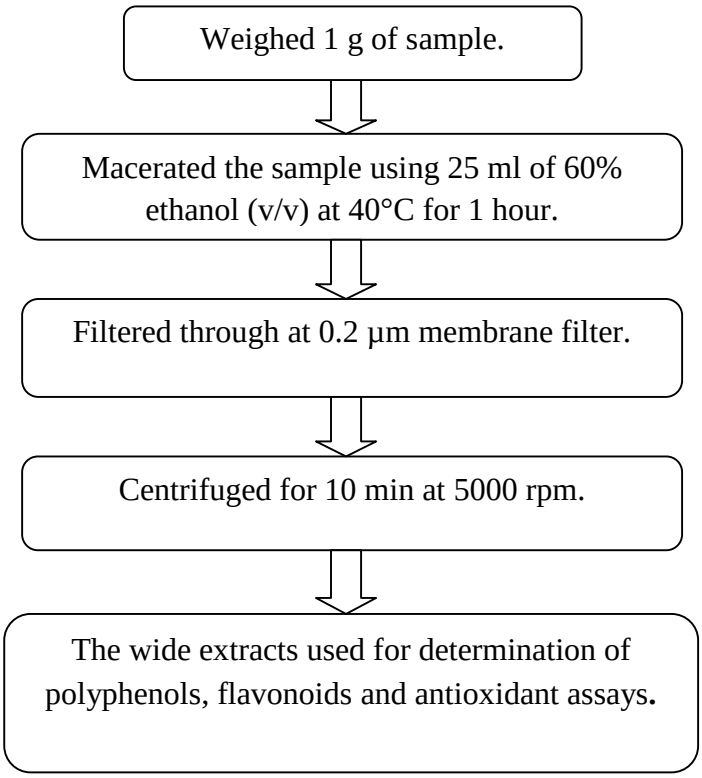


Figure 15: Maceration process **(Bernatoniene *et al*., 2016).**

The extraction yield was calculated as follows: $\text{Yield (\%)} = (W1/W2)\times 100$

where W1 was the weight of extract after concentration and W2 was the weight of the empty beaker.

II.6. Determination of polyphenols

II.6.1.Total phenolic content

The amount of total phenolic (TPC) in the extracts was determined using Folin-ciocalteu method **(Singleton et Rossi, 1965).** Oxidation of phenolic compounds with this reagent includes reaction with the mixture of $H_3PW_{12}O_{40}$ acids in the alkaline medium (**Fig.16).**At this reaction a mix of blue oxides is formed **(Lapornik *et al*., 2005).**

- **Method**

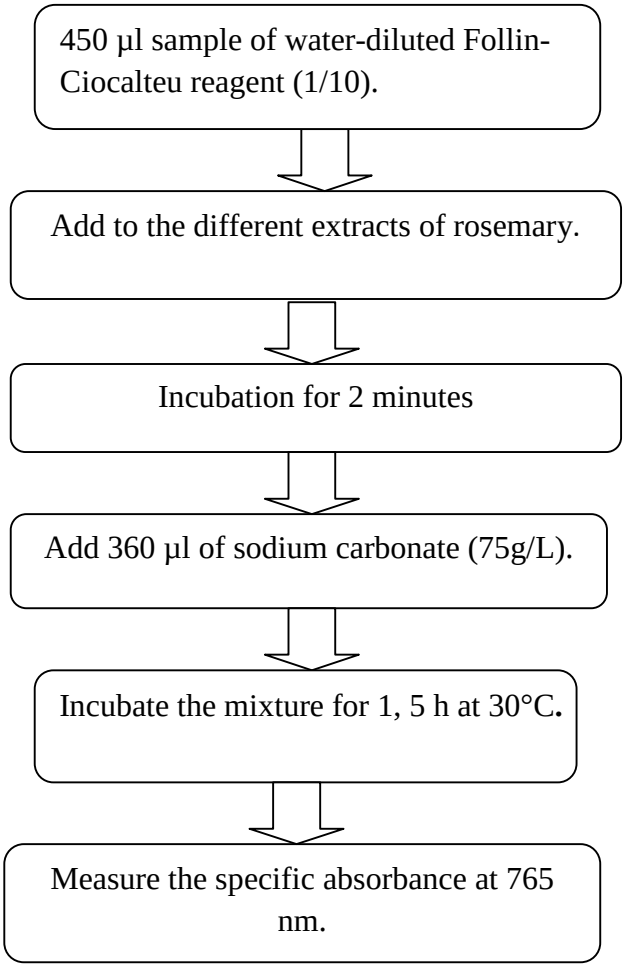


Figure 16: Total phenolic content **(Singleton et Rossi, 1965).**

The absorbance was measured by Uv-vis light spectrophotometer (spectro Scan 50, United Kingdom). TPC concentration was calculated from a calibration curve, using Gallic acid as a standard and the results were expressed as mg Gallic acid equivalents by g of dry matter (GAE/ g DW). All determination was carried out in triplicate.

II.6.2. Total flavonoids content

The total flavonoid content (TFC) was determined according to the mostly applied colorimetry method based on the formation of aluminium-flavonoid complexes **(Ribéreau, 1968)** and following the procedure **(Quettier *et al*., 2000) (Fig.17).**

- **Method**

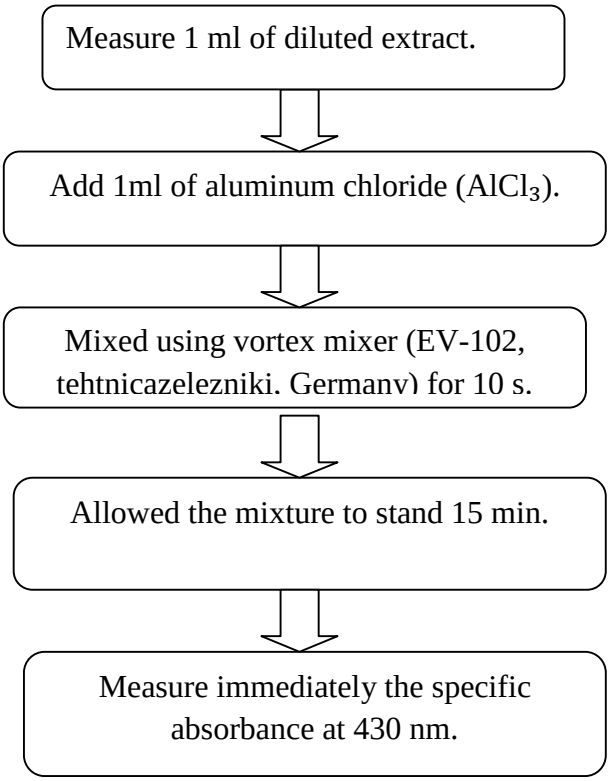


Figure 17: Total flavonoids content **(Quettier et *al.* 2000).**

The absorbance was measured by Uv-vis light spectrophotometer (spectro Scan 50, United Kingdom). TFC concentration was expressed as mg Quercetin equivalent per g of dry matter (QE/ g DM). Samples were measured in triplicate.

What was planned to be done:

-Extraction procedures of essential oil.

- Extraction of polyphenols from oils.

-Antioxidant assays.

-Soybean oil enrichment.

a- Preliminary study

b- Experimental design: Theory and application

-Heating conditions.

-Sensory evaluation.

III. Results and discussion

III.1. Moisture content

The result showed that leaves and flowers of *Rosmarinus officinalis* have moisture content of 55%, which correspond to 45% dry matter (DM).

Drying process involves removal of unbound (free) moisture from the surface first and later bound moisture from the interior of the food product till a defined limit is reached. It involves simultaneous heat and mass transfer operations. During these operations, the agricultural product is fully exposed to drying conditions of temperature and relative moisture of air, thus improving the drying operation **(Babu *et al*., 2018).**

Albu *et al*. (2004), obtained either in bulk or in a dried form (containing about 5% by weight moisture) or as fresh leaves (containing 40% by weight moisture), which are different with our results. The difference in the levels of water in our samples compared to those of previous work may be due to drying conditions; drying time and spread thickness. Relative humidity and moisture isotherm of air must all be controlled for ensuring desired final moisture quality.

III.2. Extraction procedures of polyphenols

III.2.1. defatting yield

Delipidation was used to dearomatize the powder of rosemary. This sample was defatting using the Soxhlet apparatus, using petroleum ether as solvent because it contains fat; which is expressed as a percentage (%). The defatting removes not only fats, but also aromatic compounds of different origins **(Bou-Maroun et Cayot, 2011).**

After carrying out the step of delipidation we have calculated its yield about 0.32 % **(**delipidation yield).

Based on the results obtained, the high amount of oil was 0.13% in the leaves of rosemary and this amount of oil is considered higher than the oil in other geographical locations in the same region (0.133%), It is known that the variability of the DY content of a plant depends on environmental factors such as harvest time, soil, climatic conditions and other factors **(Amani *et al*., 2018).**

According to the results obtained by **Amani *et al*., (2018)** the pale yellow oil isolated from rosemary leaves was 1.8% w/v , it was composed of 23.04% camphor, 14.01% 1,8-cineole and 13.8% terpinen-4-ol., In general, the diversity of rosemary oils amounts. oils in different locations was spectrometrically attributed to many factors, including the geographical environment, gene pool, plant population density in addition to the physical and chemical characteristics of the soil, growing medium and time of picking.

III.2.2. Extraction yield

To select the best method for polyphenol yield extraction rosemary, conventional maceration (CM), microwave assisted extraction (MAE) and Ultrasound-assisted extraction (UAE) were used. The mean values of yield extraction of rosemary plant are shown in **(Fig.18)**. The highest extraction yield was obtained with MAE (10.5±0.0017%), whereas the extraction yield with the rest of methods extracted less material (UAE: 9.75±0.0003%; CM: 7.14±0.0004%) at $p<0.05$.

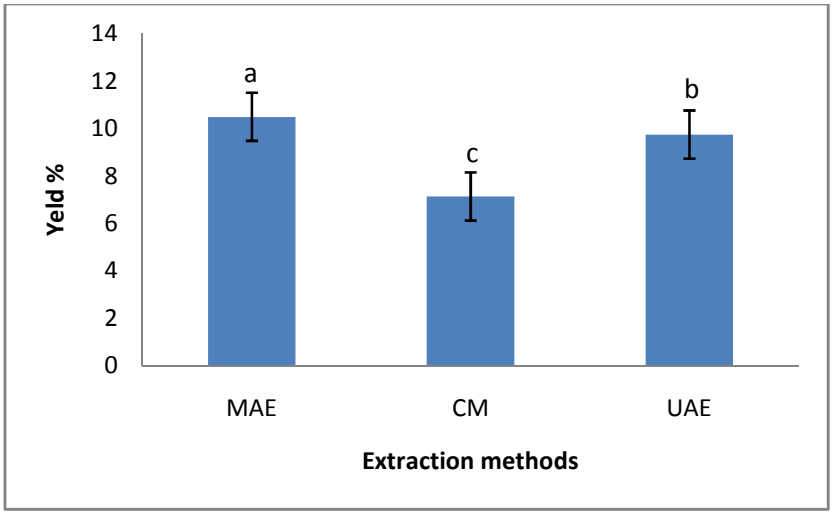


Figure 18: Yield extraction phenolic compounds from rosemary leaves extracts.

The results of extraction yields of this study are different from those reported in the literature (**Tab.IX**).The rosemary leaves were subjected to conventional maceration for 1 hour, the extraction temperature was 40°C using a 60% (v/v) of ethanol as solvent, with a result of 7.14% which was lower than that of **Jacotet-Navarro. (2015)** who used ethanol/water (90/10, v/v) for 30 min, it can be seen that this difference may be due to the increase in the concentration of the solvent (polarity). Compared to the work of **Rodríguez-Rojoet *et al.* (2012)** using 96% ethanol for 15min in 40°C, our result is higher; the difference is may be due to the extraction time even to the increasing the proportion of water of solvent.

Table IX: Comparison of total yields contents of rosemary with different methods of extractions

Extraction methods	Yields of our experiments	Yields of other works	References
CM (Ethanol 60%)	7.14±0.0004%	10.0±0.3% (Eth/40°C/30 min)	**(Jacotet-Navarro, 2015**).
		2.4±0.2 Eth/40°C/15min	**(Rodriguez-Rojo *et al*., 2012)**
		8.98±0.09 %(eth60%)	**(Ankik et Chaouati, 2019)**
UAE (Ethanol 56%)	9.75±0.0003%	13.1 ± 0.1%(eth)	**(Jacotet-Navarro, 2015**).
		17.75%±1.20%(water)	**(Hosseini *et al*., 2018)**
		10.09±0.03% (eth56%)	**(Ankik et Chaouati, 2019)**
MAE (Ethanol 70%)	10.5±0.0017%	25.2% (eth).	**(Jacotet-Navarro, 2015**).
		19% (eth70%)	**(Bellumori *et al*., 2016).**
		10.71±0.03% (eth 70%)	**(Ankik et Chaouati, 2019)**

However our extraction yield values using the three methods are much closer than those found by **Ankik et Chaouati (2019)**, who used the same optimal extraction conditions, this slight difference of the results obtained is probably due to the region and the harvest period of the plant matrix and also they used an industrial drying process unlike us we dried our sample in a drying oven.

Comparing to **Rodríguez-Rojo *et al*.(2012**) using ethanol 96% for 15min in 40° our result is higher, the difference is due to the time of extraction even the high concentration of the solvent.

Jacotet-Navarro.(2015) used ethanol/water (90/10, v/v) as solvent with 1 KW a power of ultrasound for 30min at 40±1°C, our result is lower thanthis result, this deference is due to the higher concentration of the solvent and the extraction time, but also to the high power of the ultrasound which allowed a higher extraction yield.

According to **Hosseini *et al*.(2018),** the yields of the extract from the water process (sonication power 125 watts) carried out with 100% water for 7.5 minutes from rosemary

dried in an oven at 40°C for 24 hours and dried again for 24 hours at 27°C in the dark were 17.75%±1.20%, which is higher than our result.

The efficiency of MAE in this extraction was evaluated, an extraction time of only 5 min was used and 70% of ethanol as solvent with 800W of microwave power, compared to other researches, our result is the lowest.

According to **Jacotet-Navarro.(2015)**, microwaves with a power of 210 W and ethanol (90/10, v/v) as solvent for 30 minutes at 78°C, our result is much lower, which is due to the concentration of the solvent and the extraction time.

Bellumoriet *et al*. (2016), used 70% of ethanol for 10 minutes, our result is lower than thier result due to the extraction time which is an important parameter, even though we used the same solvent concentration.

We find that the best method is microwave extraction, compared to conventional extraction methods, microwave extraction can take much less time; it lasts about 30 min or less, which considerably reduces the time needed for extraction, which is a very important factor in analytical chemistry applications (**Routray et Orsat,2012).**

Many of the effects are associated with ultrasound processing are attributed to the phenomenon of cavitation which promotes agitation and temperature increase, which may promote the extraction of polyphenols by increasing diffusion and solubility; however, the use of ultrasound at mild temperatures (< 40°C) to obtain extracts from Mediterranean plant matrices is limited **(Munekata *et al*., 2020)**,

Comparing our results with other researches, some are higher and others lower. We found that this difference may be due to severol factors such as the type of solvent, its percentage, the deference in the region, the harvest period of the plants and perhaps also the application of the solvent; according to **Routray et Orsat. (2012)** the temperature and the extraction time also affect the extraction yields.

III.3. Phytochemical analysis

III.3.1.Total phenolic compound

The content of total phenolic compounds was estimated using the Folin-Ciocalteu method, it was determined directly from the gallic acid calibration curve which has a regression coefficient close to 1 ($R^2 = 0.999$)..

The **(Fig.19)** depicted the amount of total phenolic contents (TPC) of *Rosmarinus officinalis*, using different extraction methods. Polyphenols contents presented significant differences according to the used technique.

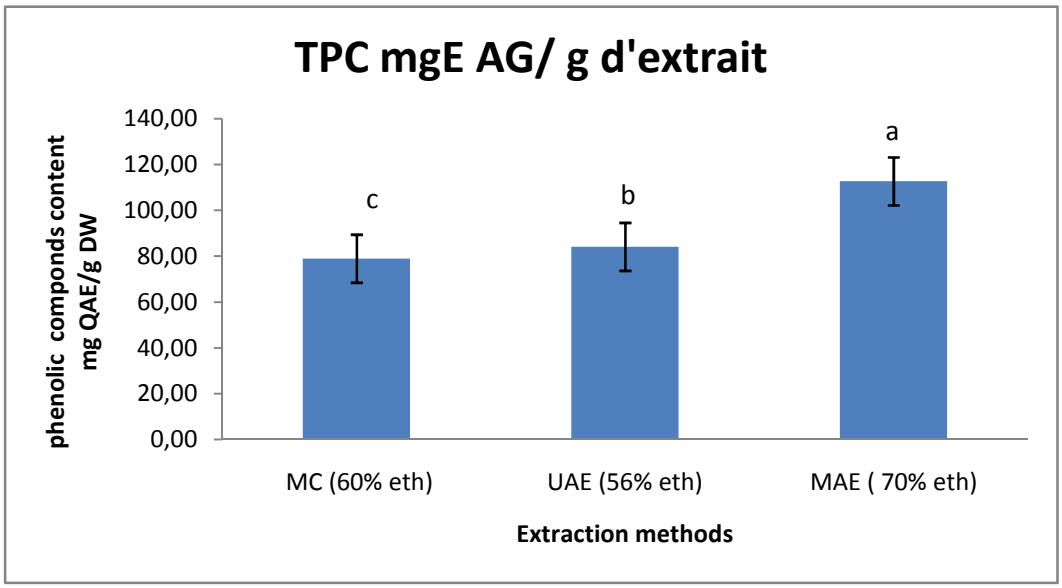


Figure 19: Polyphenol content of aerial part of Rosemary.

The highest level of TPC has been detected in MAE, followed by UAE and CM. Thus, the microwave extraction, represent the best method for the extraction of rosemary polyphenols.

The table bellow showed our results of TPC and the results of others researches.

Table X: Comparison of TPC using different extractions method

TPC of our experiments		TPC of others works	References
CM (Ethanol60%)	78.98±0.007 mg/g of extract DW	212.5 mg / g (eth 60%)	**(Kasparavičienė *et al.*, 2013)**
		24 mg / g (eth 50%).	**(Munekata *et al.*, 2020)**
		25 mg/ g (water 100%).	**(Munekata *et al.*, 2020)**
		180 mg/g of extract DW (etha56%).	**(Ankik et Chaouati, 2019)**

UAE (Ethanol 56%)	84.15±0.013mg/g of extract DW	199.07 mg GAE/g of extract DW (eth 56%). 20 mg/ g (eth 50%). 18 mg/ g (water 100%). 190 mg GAE/g DW (eth 56%).	**(Hosseini *et al*., 2018)** **(Munekata *et al*., 2020)** **(Munekata *et al*., 2020)** **(Ankik et Chaouati ,2019)**
MAE (ethanol 70%)	112.70±0.021mg/g of extract DW	32.9 mg GAE/g dried extract (eth 70%). 162 mg GAE/ g (eth) 210 mg GAE/g DW (eth 70%)	**(Bellumori *et al.*, 2016)** **(Erkan *et al*., 2008)** **(Ankik et Chaouati, 2019)**

Ankik and Chaouati, (2019), have estimated the TPC of rosemary from Bejaia, they found very high TPC, (CM: 180mg GAE/g of Dw, UAE: 190 mg GAE/g Dw and MAE=210 mg GAE/g of Dw) under the same extraction conditions, this variability in phenolic content depends on environmental factors such as region and harvesting time of the plant matrix and also they used an industrial drying process unlike us we dried our sample in a drying oven.

Hosseini *et al*. (2018) found a total polyphenols content equal to 199.07 mg GAE/g of extract DW, this high content was obtained under optimal conditions 12.60 min, 200w and 56% ethanol, these values were better than those recorded in the previous study. This difference may be related to extraction power and temperature; in general, higher amounts of polyphenols are extracted when a longer extraction time is used, while polyphenols degradation may also occur when longer processing times are associated with higher temperatures. Therefore, a longer extraction time resulted in a lower polyphenols extraction rate and was in agreement with the literature **(Wong *et al*., 2017)**. The application of a solvent containing both water and ethanol facilitates the extraction of polyphenols from plant material. Water swells the plant material, allowing ethanol to penetrate solid matrices more easily to disrupt the binding between the plant matrixes and facilitate better mass transfer **(Hosseini *et al*., 2018).**

The total phenolic content obtained in the present study is higher than the values described by **Munekata *et al*. (2020)** who used two extraction methods with 50% ethanol and 100% water and found 25 and 24 mg GAE/g dry matter respectively for conventional maceration. In addition, the total phenolic compounds in the ultrasonically extracted samples were described as 20 and 18 mg GAE/g dry weight, respectively. These values may be due to the difference between our optimal extraction conditions and theirs. Such as extraction ration and total extraction volume, temperature, time and extraction power, the use of a mixture of water and

ethanol as solvent can facilitate the extraction of water and/or ethanol soluble solids. This explains why the TPC of aqueous ethanol was higher than the TPC of water.

Kasparavičienė *et al*. (2013), found a higher phenolic contents (212.5 ± 4.39, mg GAE /g dry weight) than those obtained in the current survey. This difference could be due to climatic conditions, harvest time and cultivars**.** Indeed, the extraction conditions in terms of temperature and number of extraction steps as well as the condition and origin of the sample in terms of geographical provenance cannot be excluded **(Li *et al*., 2009)**.

The values recorded in our study are significantly higher than those obtained by **Bellumori *et al*. (2016)** using microwave (32.9 mg GAE/g Dw) and same solvent (70% ethanol) both plant/solvent ratio (1 g dried leaves/10 ml), and the extraction time (10 min) were different. Our results show that the best extraction process for TPC is MAE in ethanol.

The results of our work can be used as a basis for future developments, design and practical recovery of rosemary phenols**.**

So Solvent choice clearly plays a major role in MAE, where ethanol and water mixture were found to be the most suitable solvents for guaranteeing high total phenolic compound recovery from dried rosemary leaves **(Bellumori *et al*., 2016).**

Then we also see that the results of **Erkan *et al*. (2008)** and **Galan *et al*. (2017)** are higher than our results due to the time of extraction we extracted for only 5 min while they remained the extraction procedure for 2 hours**.**

III.3.2. Total flavonoids

The determination of flavonoids was carried out according to the AlCl3 method using quercetin as standard the flavonoids contents are expressed in mg QE/mg extract.

The amount of total flavonoids (TFC) of *Rosmarinus officinalis,* was determinate using different extraction methods, MAE and UAE and maceration (CM).The TFC results of rosemary extracts are shown in **(Fig.20).**

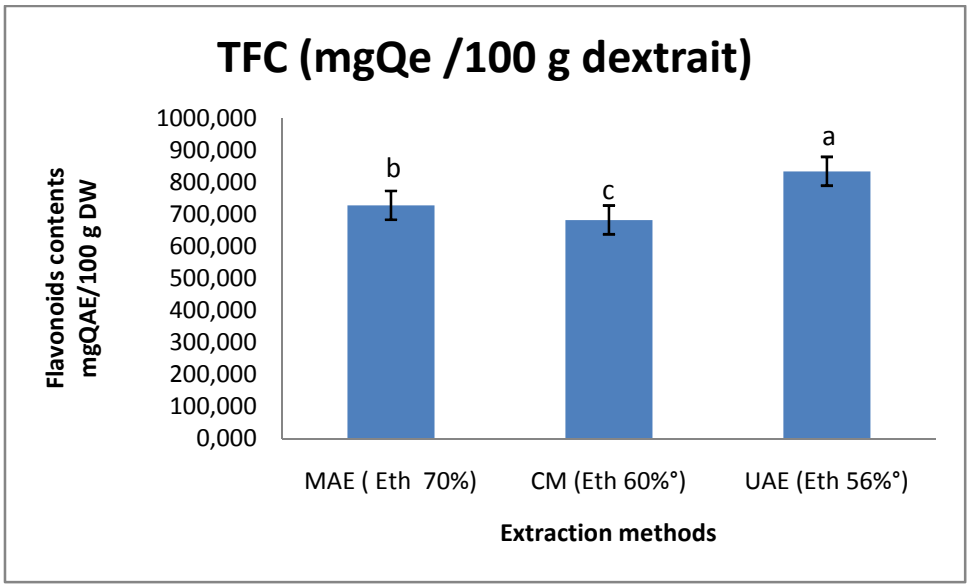


Figure 20: Flavonoids content of aerial part of rosemary.

The highest level has been detected in UAE (834.571±0.014 mg QE/100g extract), following by MAE (728.342±0.025 mg QE/100g extract) and CM (682.62±0.009 mg QE/100gextract).

Flavonoids contents of different works, results of this study and those of others researches, are given in the table XI

Table XI: Comparison of total flavonoids contents using different extractions method

TFC of our experiments		TFC of other Works	References
CM (Ethanol 60%)	**682,62±0,01mgQE/100g Dw**	2123mg CE/100 g DW (water) 1522 mg CE/100g DW (Eth50%) 7550±3 mg rutine/100g extract	**(Munekata *et al.*, 2020).** **(Munekata *et al.*, 2020).** **(Ankik et Chaouati., 2019)**
UAE (Ethanol 56 %)	**834.57±0,01mg QE/100gDw**	2499 mg CE/100g DW (eth). 15.5 mg/100g DW (ace). 8540±0.2 mg RuE/100g extract (eth60%)	**(Munekata *et al.*, 2020)** **(Bellumoriet *et al.*, 2016).** **(Ankik et Chaouati., 2019)**

MAE (Ethanol 70%)	**728.34±0,02mg QE/100gDw**	352.5mg/100g DW (ethanol 70%) 18mg/100g(ethanol70%.) 8730±0.06 mg RuE/100g extract	**(Švarc-Gajić *et al.*, 2013). (Bellumori *et al.*, 2016). (Ankik et Chaouati,2019)**

CE: Catechine equivalents RuE :rutine equivalents DW : dried weigh

The TFC detected in this survey are close to those found by **Ankik et Chaouati. (2019)** that have obtained in MAE: 8730±0.06 mg RuE/100g extract), following by UAE (8540±0.2 mg RuE/100 extract) and CM (7550±3 mgRuE/100g extract) under the same extraction conditions, this slight difference in TFC may be due on environmental factors such as region and harvesting time of the plant matrix and also they used an industrial drying process unlike us we dried our sample in a drying oven.

Our TFC values are higher than those found by **Munekata *et al*. (2020)**:15.22mg CE/g DW and 24.99mg CE/g DW for extracts obtained by maceration and ultrasound with the same solvent (56% ethanol) respectively. This difference probably due to the extraction ration, temperature, time and extraction power.

According to the results of **Švarc-Gajićet *et al.* (2013),** the rosemary extract obtained by microwave in the same solvent concentration (70% ethanol) and the same extraction time (5 min) is almost half of the value found in this work. This difference can be attributed to extraction conditions (power = 320 w and extraction ratio = 5g/25 ml).

Chemat *et al*.(2017) and **Routray et Orsat.(2012)** reported that several influencing factors the MAE such as: Power and frequency, intensity, shape and size of ultrasound, effect of Microwave Power Level, effect of solvent system which is one of the most important factors in an extraction process, effect of Temperature witch impacts the solvent's properties, effect of time application of extraction, effect of contact surface area; when the contact surface area of the plant material increases, then the extraction efficiency generally increases**.**

According also to **Bellumori *et al.* (2016)** solvent choice clearly plays a major role in UAE and MAE, where ethanol and acetone were found to be the most suitable solvents for guaranteeing high total phenolic compound recovery from dried rosemary leaves. It is well known that the quality of an extraction from a plant material strongly depends on the degree of isolation of the analytics substances from vegetable matrix (**Routray et Orsat,2012).**

We also notice that TFC follow the same tendency of TPC. This can be explained by the relationship between TPC and TFC; positive correlation between these two classes of polyphenols. A significant relationship between these two variables, indicating that flavonoids were the main polyphenols group in rosemary.

The aim studied in this work is elaboration of oil with added value and the main objective in this study was the enrichment of soybean oil with phenolic compounds and essential oil from rosemary leaves and flowers, but due to this pandemy we have not been able to continue the work. Thus, the obtained results revealed that:

- The moisture content of leaves and flowers of *Rosmarinus officinalis* found is 55% which correspond respectively to 45% dry matter (DM).

-The values of polyphenols extracted with microwave assisted extraction under optimal conditions (output power of 800 W, 5 minutes) using 70 % ethanol were higher than those with ultrasound assisted extraction (130 W, 12 min) using 56% ethanol and with conventional extraction (40 °C, 1H) using 60% ethanol. The MAE has recorded the highest yield value (10.5 ± 0.0017), whereas the extraction yield with the rest of methods extracted less materials (UAE: $9.75 \pm 0.0003\%$; CM: 7.14 ± 0.0004.) at $p<0.05$.

- The highest amount of total phenolic compound was (112.70±0.021 mg GAE/g of extract) obtained by the microwave assisted extraction than ultrasound (84.15± 0.013 mg GAE/g of extract) and maceration (78.98±0.007 mg GAE/g of extract). Where by the flavonoid contents; UAE>MAE>CM; 8.35±0.007 mg QE/g of extract; 7.28±0.01 mg QE/g of extract; 6.83±0.009 mg QE/g of extract respectively.

Perspectives

- Extractions of rosemary essential oil
- Enrichment of vegetable oils with polyphenols and essential oil of rosemary
- Quantification and identification of phenolic compounds and essential oil by HPLC, GC/MS
- Evaluate of biological activities of rosemary extracts and enriched

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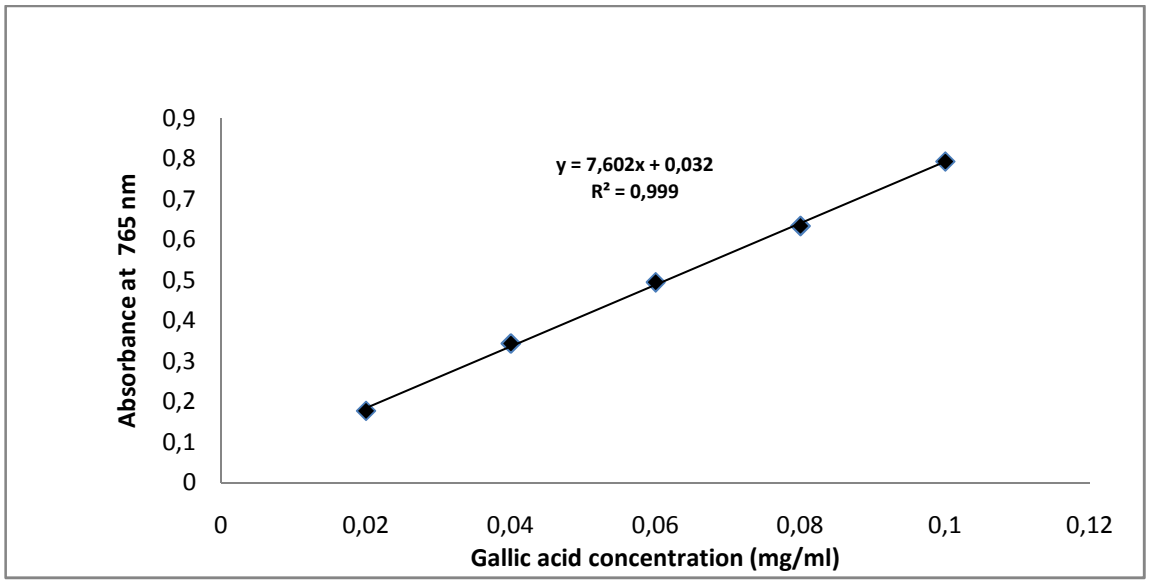
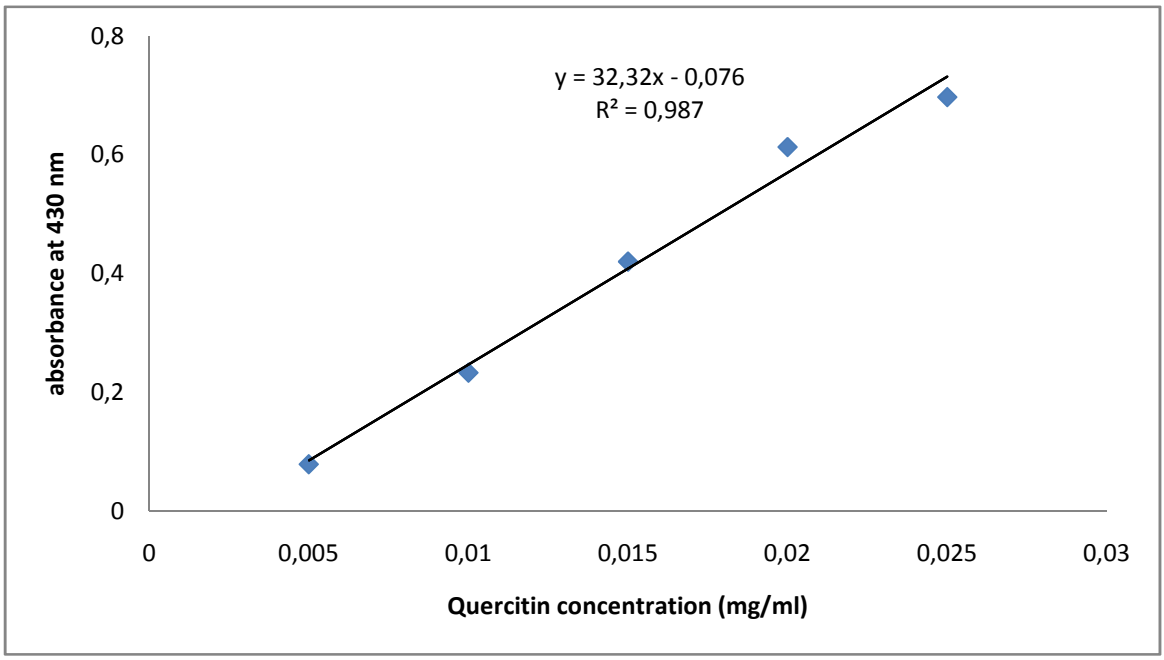
Appendix

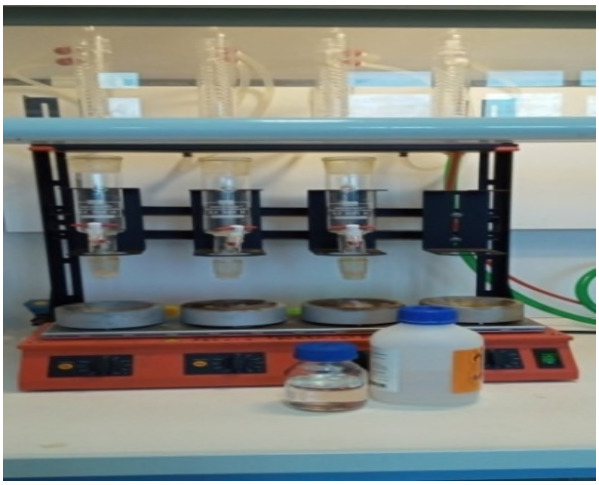
Appendix I: Gallic Acid Calibration Curve



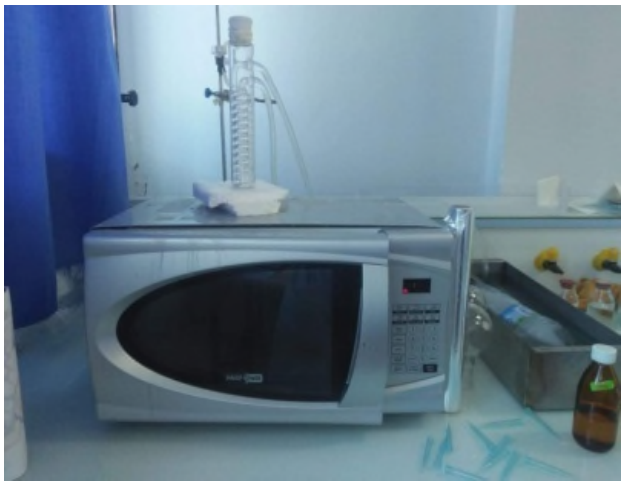
Appendix II : Quercitin Calibration Curve



Appendix III



Soxhlet apparatus



Microwave apparatus

Ultrasound apparatus

Abstract

The aim of our work is to enrich soybean oil with polyphenols and essential oils extracted from the leaves and flowers of *Rosmarinus officinalis* and to enhance their antioxidant capacity, but due to the pandemic and quarantine, we were unable to carry this out. In this study, we used three methods: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and conventional maceration (CM) to extract the polyphenols of rosemary from the aerial part of the plant; the best method was MAE, allowing to obtain a better extraction yield and a better total polyphenols content which are respectively: 10.5±0.0017%, 112.70 mg GAE /g DW extract. Just as the content obtained by UAE is 834.57 mg QE /100g DW for flavonoids, the comparison of our results with other research has shown that the differences are due to several parameters such as time, temperature, nature of solvents and its concentration, type of drying, location of harvest.

Keywords: polyphenols, essential oils, rosemary, microwave assisted extraction, ultrasound assisted extraction

Résumé

Le but de notre travail est d'enrichir l'huile de soja avec des polyphénols et des huiles essentielles extraites des feuilles et des fleurs de *Rosmarinus officinalis* et d'améliorer leur capacité antioxydant, mais en raison de la pandémie et de la quarantaine, nous n'avons pas pu y parvenir. Dans cette étude, nous avons utilisé trois méthodes : l'extraction assistée par micro-ondes (EAM), l'extraction assistée par ultrasons (EAU) et la macération conventionnelle (MC) pour extraire les polyphénols du romarin de la partie aérienne de la plante ; la meilleure méthode était la MAE, permettant d'obtenir un meilleur rendement d'extraction et une meilleure teneur totale en polyphénols qui sont respectivement : 10,5±0,0017%, 112,70 mg d'EAG /g d'extrait. Tout comme la teneur obtenue par les EAU est de 834,57 mg QE /100g d'extrait pour les flavonoïdes, la comparaison de nos résultats avec d'autres recherches a montré que les différences sont dues à plusieurs paramètres tels que le temps, la température, la nature des solvants et leur concentration, le type de séchage, le lieu de récolte.

Mots clés : polyphénols, huiles essentielles, romarin, extraction assistée par micro-ondes, extraction assistée par ultrasons.

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