République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université A. MIRA - Béjaia

Faculté des Sciences de la Nature et de la Vie
Département des Sciences Alimentaires
Spécialité : Qualité des Produits et Sécurité Alimentaire

Réf :.....

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

MASTER

Thème

Effet d'incorporation de l'huile essentielle de Romarin sur la qualité de l'huile de soja

Présenté par :
AICHE Arezki & BOUBAYA Lyes
Soutenu le : **02/07/2019**

Devant le jury composé de :

Mr KHODIR Madani	Professeur	Président
Mme ACHAT Sabiha	MCA	Encadreur
Mr MOKRANI A/Rahman	MCB	Examinateur
Mr ALIANE Khellaf Ingénieur	R&D	Invité

Année universitaire : 2018 / 2019

ACKNOWLEDGMENTS

FIRST AND FOREMOST, MY MOST EARNEST GRATITUDE TO OUR LORD, ALLAH, FOR BLESSING US WITH THE ABILITY TO UNDERTAKE THIS STUDY AND GRANTING US THE STRENGTH TO COMPLETE IT.

Foremost, we would like to express our sincere gratitude to our advisor ***Dr. Sabiha ACHAT*** *for the continuous support of our study and research, for her patience, motivation, enthusiasm, and immense knowledge.*

A sincere gratitude to our sub advisor at Cevital group ***Mr. Aliane khellaf*** *his guidance helped us in all the time of research and writing of this thesis.*

We could not have imagined having better advisors for our study.

Our sincere gratitude to the R&D laboratory at "Cevital Group" especially Mr Hedjal
We would also like to thank all the members of our thesis committee for taking their valuable time to come and carefully evaluate our manuscript

Prof K.MADANI and Mrs. MOKRANI

We would like to thank the ***L3BS*** *team and all of the wonderful Doctorants, teachers and all engineers Sonia, Nabila, Farida, Amina, Khadidja,, Khokha, Melissa, Abd el Ghani, Mekhlouf Mme Karima Debbou*

DEDICATION

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.

Mom and Dad, you have given me so much, thanks for your faith in me, and for teaching me that I should never give up. Thanks for lending me your ear on countless occasions when I needed to vent my frustrations…

To my sister Lamia, you have always been there for me with encouraging words.

To my wife Fadila, Thank you for your love and support, you have always been there for me with encouraging words and understanding without you I will never succeed after 14 years to come back to study.

To my dear samira who encourage me and say always I will be like you.

To my binomial lyes, and all QPSA section with whom I shared good times during this year.

To my dears, sun and daughter Walid and myriam hope that you will find this work at the university in the future.

Arezki

DEDICATION

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.

Mom, you have given me so much, thanks for your faith in me, and for teaching me that I should never give up. Thanks for lending me your ear on countless occasions when I needed to vent my frustrations …
Father, you have always been there for me with encouraging words
Thank you for your love and support.
To the best brother in the world Omar, Raouf and Fadi
To my Sisters that I love very much Nihad and Bariza

To my girlfriend Nora you are everything for me, thanks for your patience, encouraging words and understanding

To all my familly: grandfathers and grandmothers, my uncles and aunts, cousins and cousins
Especially my uncles Fatah

To my binomial and friend arezki with which I have division a good moment and her family
To all my friends Halim,Tayab, Nacer, Abd Elnour, Djamel and Adel

Lyes

List of abbreviations:

ANOVA: Analysis Of Variance

°C: Celcus degrees

DPPH: 2, 2-Diphenyl-picrylhydrazyl

GAE: Gallic Acid Equivalent

MAE: Microwave Assisted Extraction

G: gram

H: hour

Min: minute

ML: milliliter

RSA: Radical Scavenging Activity

s: second

UAE: Ultrasound Assisseted Extraction

V/v: volume/ volume

W: Watt

REO: Rosemary Essential Oil

IC_{50}: Inhibitory Concentration

$EC_{50:}$ Effective Concentration

US: Ultrasound

ppm: parts per million

SO: Soybean Oil

HD: Hydrodistillation

US/MAHD: Ultrasound assisted microwave hydrodistillation

Lists of tables:

List of figures:

SUMMARY

Summary

CHAPTER III: RESULTS AND DISCUSSION

INTRODUCTION

Edible vegetable oils such as soybean oil, sunflower oil, and olive oil hold an important place in human nutrition. These oils are consumed as salad oils, cooking oils, or frying oils. Soya oil, produced from the glycine seeds, contains tocopherols and phenolic compounds as antioxidants. During frying, a gradual deterioration of oil occurs due to oxidative decomposition reactions. Lipid oxidation leads to the production of compounds that reduce the quality of frying oils and foods **(Salta *et al*., 2007).**

Antioxidant addition during industrial food formulations is one of the most effective means to retard fat oxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in many foods. However, their use has been questioned because of issues related to toxicity and carcinogenicity. For this reason, great interest has been given to the application of natural antioxidants in foods namely to enrich oils, because of their potential nutritional and therapeutic effects. **(Achat *et al*., 2012)**

A number of spices and herbs containing antioxidant compounds have been concentrated as extracts or essential oils. Among them, rosemary (*Rosmarinus officinalis* L.) extract has gained considerable attention as a spice with one of the most powerful antioxidant potential. This property of rosemary extract has attributed to the presence of bioactive substances; phenolic diterpene compounds, such as carnosic acid, carnosol and rosmanol, which break free radical chain reactions by hydrogen atom donation and chelating metal ions. **(Alizadeh *et al*., 2015)**

The extraction of plant material is the first and very important steps in anlytical process in various studies dealing with plant secondary metabolite. There are différent technologies that can be used for the extraction of phenolic compounds or supplementation of oils. Microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), seemed to be an effective and advanced extraction technique for bioactive compound extraction from herbal plants. Indeed, conventional maceration are either too long or demand too high temperature which causes a damage of plant material or oil **(Azmir *et al*., 2013).**

The current study propose extraction of rosemary essential oils, following by a direct enrichment of edible soya oil, with these aromatic constituents. Due to the many factors

that influence the UAE, optimization of the extraction process parameters is required to retain the maximum amount of polyphenols. In the present work, a response surface method was examined for optimization of UAE process parameters (extraction time and ratio) by employing a Box–Behnken design to maximize extraction of antioxidants from aerial part of rosemary. Several tests have been performed, as detmination of polyphenols, antioxidant capacities of the rosemary extract and processed oil (DPPH° test, ABTS and reducing power), the stability toward lipid autoxidation (heating test, Rancimat) and sensory evaluation.

In order to better situate the context of this research, a bibliography was presented on rosemary plant, antioxidant and the enrichment of edible oils.

BIBLIOGRAPHY

I.1 Overview of rosemary plant

Rosemary (*Rosmarinus officinalis*. L) is an aromatic, medicinal and condiment plant that derive from the Latin word rose (dew) and marinus (sea), which means 'dew of the sea' **(Giugnolinini, 1985**). This herb is native from the Mediterranean region and has been cultivated in many other regions **(Fig.1)**. Hence, it is widely spread in Algeria and broadly used in traditional medicine. The fresh and dried leaves of *R. officinalis* are widely used as flavors in different food products due to the powerful aromatic odor **(Zu *et al*., 2012).**

- **Vernacular names** English: Rosemary; French: Romarin; Arabic:Eklil El Djabel; Kabyle**:**Amzir or Aklil

(Goetz and Ghedira, 2012).

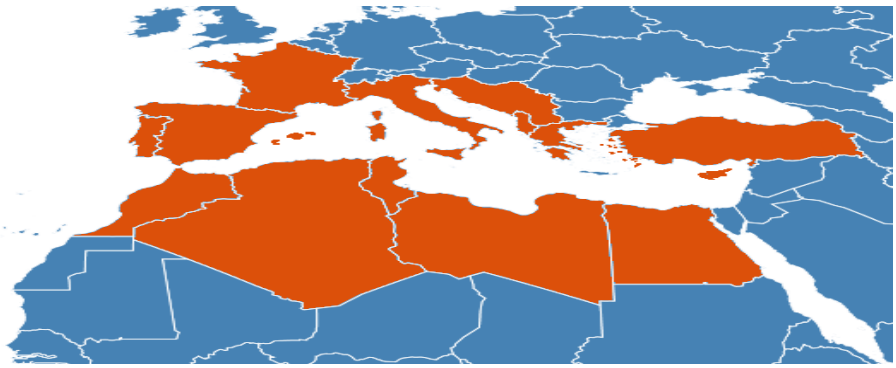


Figure 1: Distribution area of rosemary in the world

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.2).**

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

Figure 2: Taxonomic classification of rosemary**(Gaussen, 1982**)

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 and Peter, 2004).**

I.3 Chemical composition of rosemary

Rosmarinus officinalis L has an important reservoir of potential compounds, which have the advantage to be a big diversity of chemical structures possessing a very wide range of biological activities. **(Rollinger, 2004).**This plant contain lipid, sugar and high levels of vitamins (A, C) in addition to mineral element (calcium and sodium) **(Tab.I)**.**(Orhan*et al*., 2008; Švarc-Gajić *et al*., 2013).** The rosemary leaves are also quite rich in phytoconstituents namely polyphenols and essential oils. **(Rollinger, 2004).**

Table I: Chemical properties and mineral contents of rosemary. **(Orhan *et al*., 2008; 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 Essentials oils

Essential oils (EO) are defined as product manufactured by either water- or vapor distillation, by mechanical processing of citrus rinds or by steam distillation. The chemical composition and therefore the quality of essential oils can vary depending on several factors like the time of harvest, the location of the crop, the part of the plant, as well as the production method.**(Sarkic et Stappen, 2018).**EO are complex mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers). They frequently are responsible for the distinctive odor of plants. **(Nerio *et al*., 2010).**

❖ **Terpenehydrocarbons:**

Terpenes are the most common class of chemical compounds found in essential oils **(Fig.03).** They are made from isoprene units (several 5 carbon base units, C5), which are the combinations of β isoprene units, called a "terpene unit." Essential oils consist of mainly monoterpenes (C10) and sesquiterpenes (C15), which are hydrocarbons with the general formula (C5H8) n. The diterpenes (C20), triterpenes (C30), and tetra terpenes (C40) exist in essential oils at low concentration **(Mohamed *et al*., 2010).**

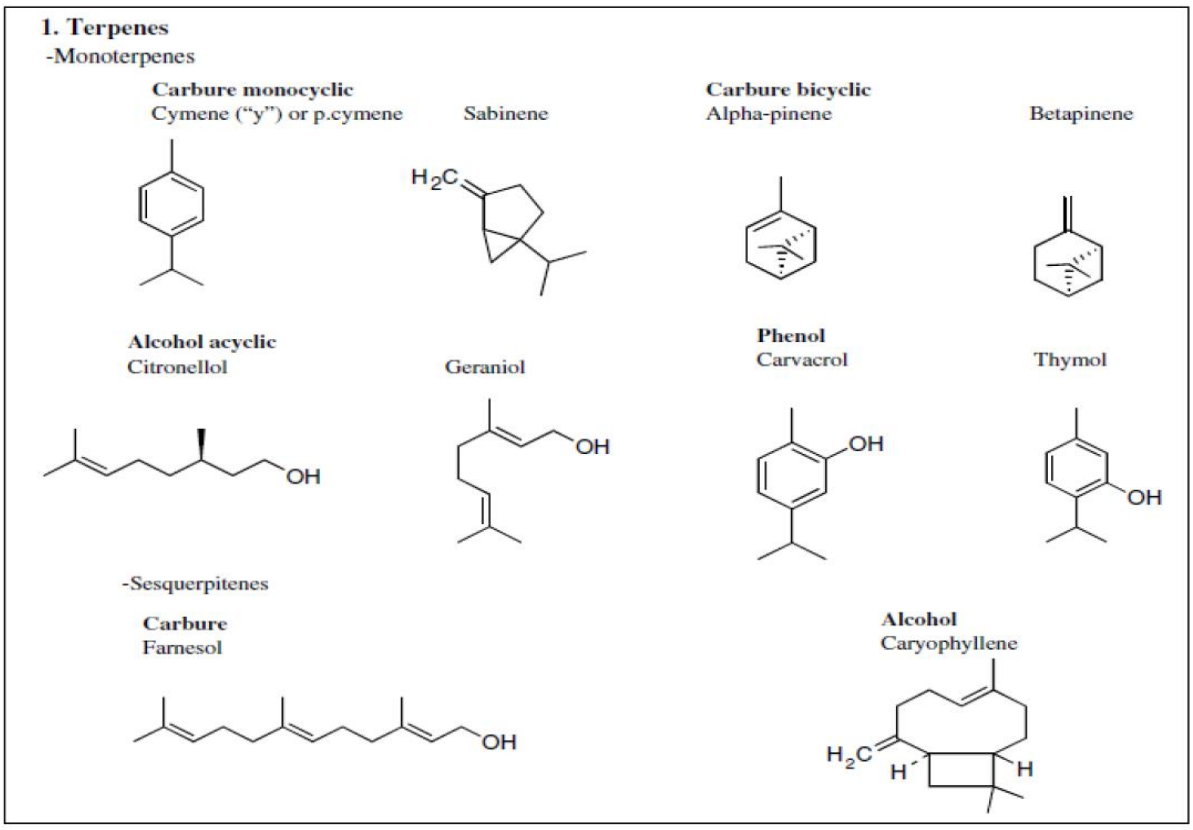


Figure 03 : Chemical structures of terpenes essential oils**(Bakkali *et al*., 2008).**

❖ **Oxygenated compounds**

Derived from phenyl propane, the aromatic compounds occur less frequently than the terpenes. These molecules are the combination of C, H, and O, and there are a variety of compounds found in essential oils **(Fig.04)**. Oxygenated compounds can be derived from the terpenes, in which they are termed "terpenoids" **(Burt, 2004).**

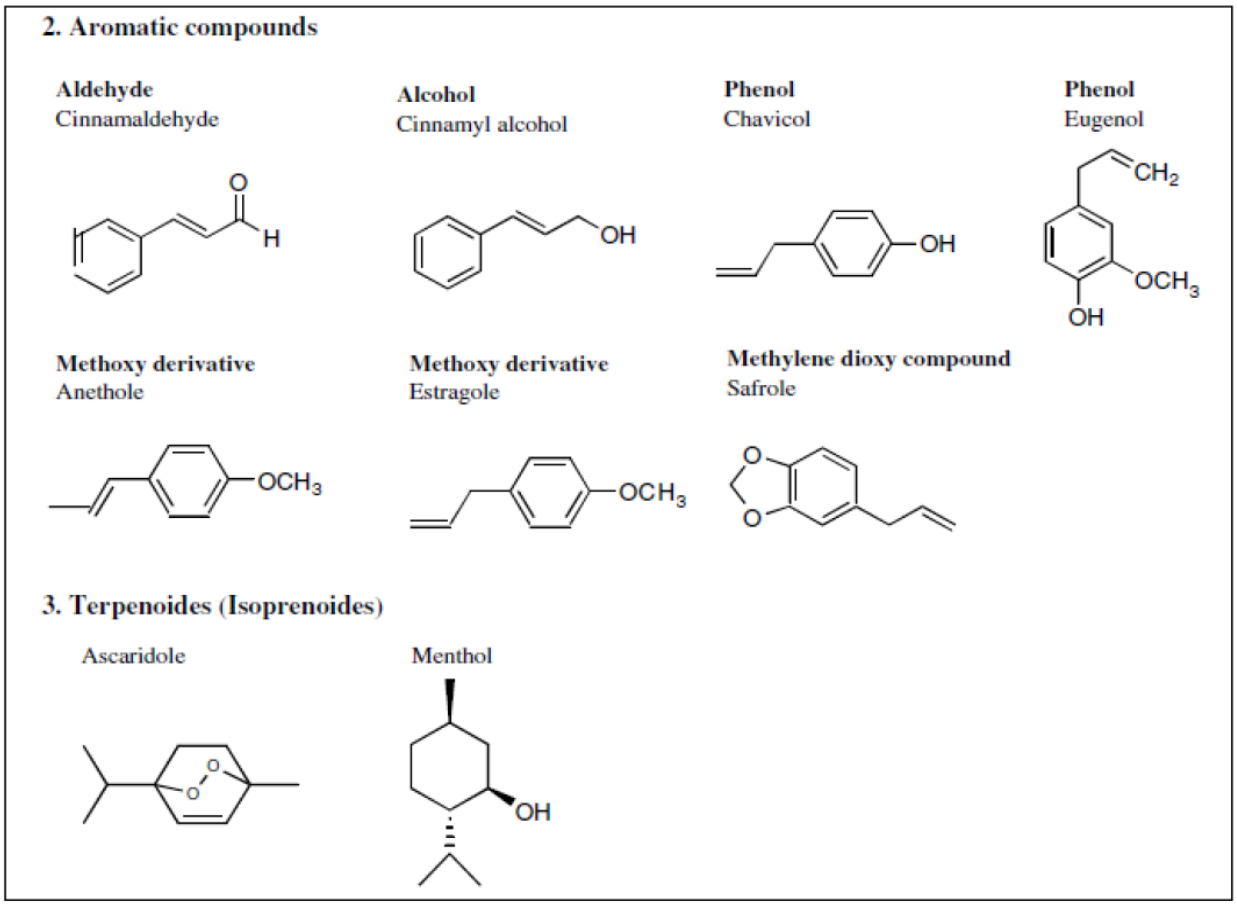


Figure 04: Chemical structures of aromatic components of essential oils **(Bakkali*et al.*, 2008**)

- The EO of rosemary are mainly phenolic diterpenes such as carnosol, carnosic acid, rosmanol and urosolic acid as shown in figure 5. **(Gad, 2015).**

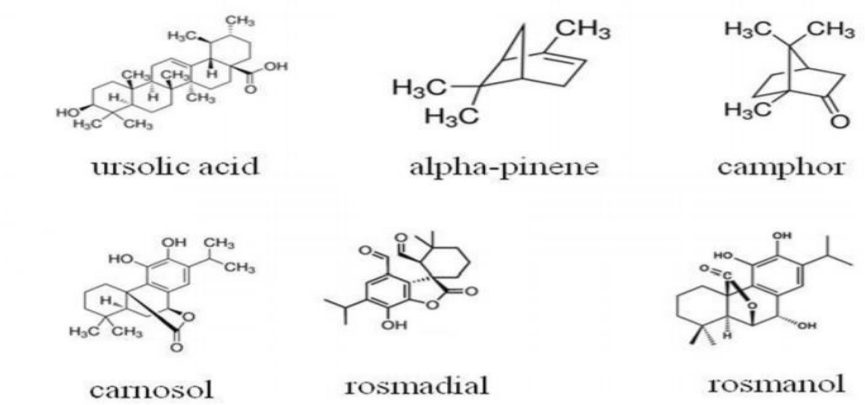


Figure 5: Structures of EO in rosemary extracts. **(De Oleveira *et al.*, 2018)**

The chemical composition of the essential oil from rosemary leaves of **(Tab. II)**, was reported using steam distillation and hydro-distillation methods

Table II: Chemical composition of rosemary essential oils

Components	Relative contents (%)	
	Steam Distillation	Hydro distillation
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.3.2 Pharmacological activities

R. officinalis L. can promote several pharmacological effects due to the interaction between the molecules of the plant and the organic systems. The effects demonstrated by this plant include ability to attenuate asthma, atherosclerosis, cataract, renal colic, hepatotoxicity, peptic ulcer, inflammatory diseases, ischemic heart disease; antioxidant and anti-inflammatory actions of rosmarinic acid ; control of hypercholesterolemia and oxidative stress and relief of physical and mental fatigue; myocardial blood pressure reduction with rosmarinic acid; antiulcer action; lipid peroxidation reduction in heart and brain; antiangiogenic and neuro protective effects of carnosic acid and carnosol; prevention of problems related to atherosclerosis; anticancer and antiproliferative effects; antiviral; and antimicrobial actions; hepato protective, nephro protective and radio protective-antimutagenic capacities; glycemia reduction; muscle relaxant and treatment for cutaneous allergy; ability to treat depressive behavior. **(De Oliveira *et al*., 2019).**

a) Antioxidant activity of rosemary

The Lamiaceae family has been a focus of the research on antioxidant compounds due to its high polyphenol content. Likewise *R Officinalis* leaves are commonly used as a condiment for flavoring food, and as a source of antioxidant compounds employed in food conservation. Antioxidants play a major role in the prevention and treatment of diseases associated with oxidative damage, including cancer, cardiovascular and neurodegenerative diseases. Reactive oxygen species, including hydrogen peroxide and free radicals, such as superoxide anion ($O^{\bullet-}$) and hydroxyl radical (HO•), are inevitably produced in living organisms resulting from metabolic processes or from external sources. 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 peroxidation through the lipid free radical scavenging mechanism .**(Andrade *et al*., 2018).**

I.4 Extraction methods of phytoconstituents

Bioactive compounds namely essential oils, can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method of extraction is steam extraction, but as technological advances are made more efficient and economical methods are being developed. These include methods such as solvent extraction, supercritical fluid extraction, ultrasound and microwave extraction. The suitability of extraction method varies from plant to plant and there are significant differences in the capital and operational costs associated **(Kabuba and Huberts, 2009).**

I.4.1 Microwave assisted extraction (MAE)

Microwaves (MW) are electromagnetic irradiation in the frequency range 0.3–300GHz (wavelengths of 1mm to 1m), between infrared radiation and radio frequencies. When microwave passes through the medium, its energy may be absorbed and converted into thermal energy. In general, the heating using microwave energy is based on two principles, ionic conduction and dipole rotation **(Zhang *et al*., 2011).**MAE can extract bioactive compounds more rapidly and a better recovery is possible than conventional extraction

processes. Thus, MAE is recognized as a green technology because it reduces the use of organic solvent **(Azmir *et al*., 2013).**

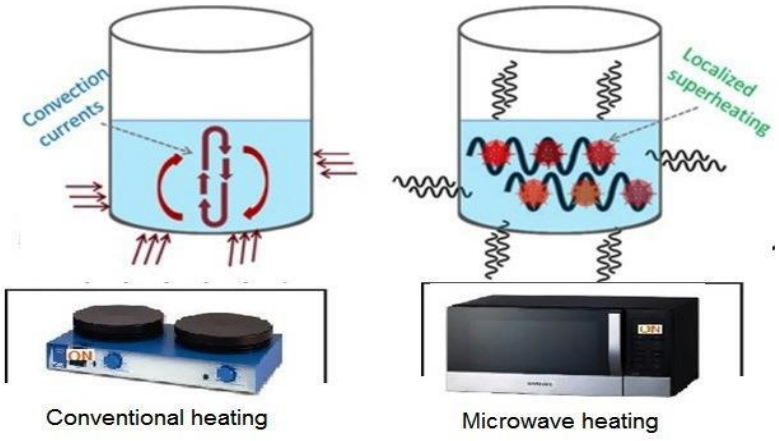


Figure 6: Conventional and microwave heating mechanisms **(Gude *et al*., 2013).**

I.4.2 Ultrasounds in Extraction processes

Ultrasound waves are similar to sound waves but have a frequency that is above 16 kHz and cannot be detected by the human ear**. (Fellows, 2000)**. Like other waves, it passes through a medium by creating compression and expansion. This process produces a phenomenon called cavitation, which means production, growth and collapse of bubbles. **(Azmir*et al*., 2013).**

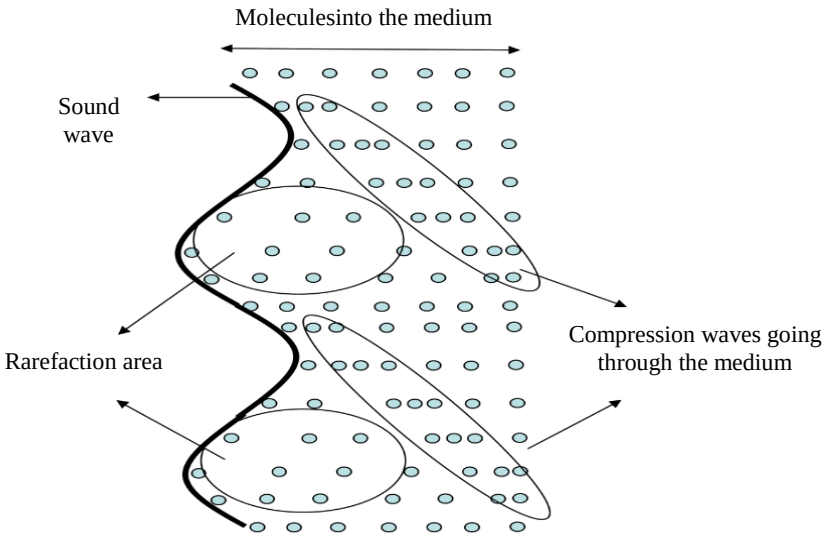


Figure 7: Compression and rarefaction cycles induced by a sound wave **(Achat*et al*., 2012).**

- The extraction mechanism by ultrasound involves two main types of physical phenomena, (a) the diffusion across the cell wall and (b) rinsing the contents of cell after breaking the walls. Furthermore, temperature, pressure, frequency and time of sonication are the governing factors for the action of ultrasound **(Herrera and De Castro, 2004).**As a consequence, employing ultrasound in the use of plant extraction has benefits in increased mass transfer, better solvent penetration, less dependence on solvent used, extraction at lower temperatures, faster extraction rates and greater yields of product**(Azmir *et al*., 2013).**

- Literature reported the use of the modern technologies MAE and UAE, in the extraction of EO rosemary plant **(Tab.III).**

TableIII: Extraction method of rosemary essentials

Methods	Experimental Remarks	Yield Extraction	Solvant	References
SupercriticalFluid Extraction	64.05 g, SC-CO2 (0.3 kg/h), 11.5 MPa, 40°C	1.03 % (w/w)	Commercial carbon dioxide (99 % purity)	**(Ivanović *et al.*, 2009)**
Conventional solvent extraction (CSE)	40 °C,4 h, 50 rpm, 1:6 (w/w)	2.5 ± 0.9 (% w/v) Etanol 0.605 ± 0.007(% w/v) water	water or Etanol 96%	**(Rodríguez-Rojo *etal*., 2012)**
Microwave-assisted extraction (MAE)	Ratio (25g) (1:6 w/w)Irradiation=(250W)t= 7 min(30s ON/OFF)	3.1 ± 1.2 (% w/v) Etanol0.095 ± 0.007 (% w/v)Water	water or Etanol 96%	**(Rodríguez-Rojo *etal*., 2012)**
Ultrasounds assisted extraction (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	**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 Etanol 96%	**(Rodríguez-Rojo *etal*., 2012)**
Hydrodistillation with a clevenger- type apparatus	Ratio (20g/200mL)(w/v)t=4hP =atmospheric pressure	23.1 ± 0.86 (mg/g)	Water	**(Liu *et al*., 2011)**

Microwave Hydrodistillation (MHD).	Ratio (20g/200mL)(w/v)power = 700 Wt=60 min	18.5 ±0.75 (mg/g)	Water	**(Liu *et al*., 2011)**
Microwave-assistedHydrodistillation (MAHD)	Ratio(350 g solvent/ 50g sample)Power=200 Wt=75 min	0.026 mL/g	distilled water	**(Karakaya *et al.*, 2014)**
hydro distillation in a Clevenger-type apparatus	Ratio (200g / 1litre)(w/v) t= 3 hours	0.86 %.	Distilled water	***(Chahboun et al., 2014)***
Microwave-assisted hydro-distillation (MAHD)	Ratio= (100 g/300 mL) t= 30 min	1.5 mL for 100 g	Water	**(Fazlali *et al.*, 2015)**
Hydrodistillation	Ratio (1:10) w/v) t= 3.5 h	1.57%	Water	***(*Marcela R *et al.*, 2 0 1 6)**
Hydrodistillation using a Clevenger-type apparatus	Ratio (100 g/ 1000 ml) t= 3 h	1.15 ± 0.102% (v/w)	distilled water	***(*Hendel *et al., 2016)***
Hydrodistillation using a Clevenger-type apparatus	Ratio (100 g / 1l) t= 240 min	1.14%	water	**(Khalili *et al.*, 2017)**
Supercritical Fluid Extraction.	Ratio (3.0 g)CO2 flow rate= 0.3-0.4 ml/minD glass beads = 2 mm	0.75(%)	Supercritical fluid	**(Khalili et al. 2017)**
Microwave assisted hydro-distillation.	Ratio (50 g/175 ml)room temperature for t=1 ht= 85 min microwave power = 888 w water volume to plant mass ratio of 0.5 ml/g	0.7756%	distilled water	**(Akhbari*et al*., 2018)**

I.5 Vegetable oil of soya

Soya oils are extracted from plant seeds of soybean: *Glycine max*, belonging to the Fabaceae family (**Rasolohery, 2007**). They are produced simply by pressing and/or solvent extraction. They are considered nonpolar and lipophilic systems whose composition is highly variable and complex, depending on their origin, quality and producing methods**. (Cossut *et al.*, 2002).**

I.5.1 Chemical composition

Triglycerides **(Fig.8)**, which are composed of three fatty acid molecules esterified to one glycerol molecule, are the main building blocks of soya oils (95–98%). The characteristics of triglycerides are determined by the types, proportions and positions of fatty acids on the glycerol backbone. According to the saturation degree of fatty acids, they can be generally classified into saturated, mono- and poly-unsaturated fatty acids, which may have different configurations, resulting in different physical and chemical properties. **(Yara-Varón *et al.*, 2017)**

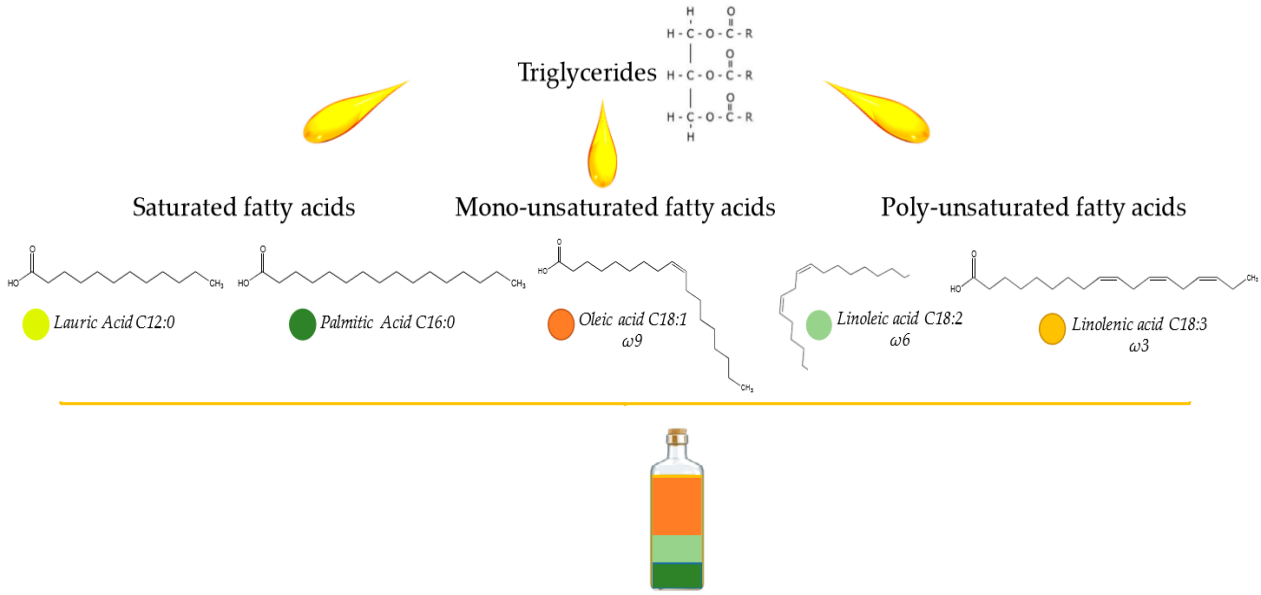


Figure 8: Major components in soya oils **(Yara-Varón et al. 2017)**

- In addition to triglycerides, the presence of minor components (less than 5%) in soya oils **(Fig.9)** cannot be ignored due to their interesting biological properties and nutritional values for pharmaceutical and nutraceutical industries. They can be divided into two types: glycolipids such as mono- and diglycerides, phospholipids, and non-glycolipids including

sterols, tocopherols/tocotrienols, free fatty acids, vitamins, pigments, proteins, phenolic compound, water, etc. Although some undesirable minor components must be removed by most consumers and food manufacturers during the refining processes **(Fig.10)**, there is no completely efficient and selective process, which will give rise to consequent colloidal structures formed by residual minor components in oils.

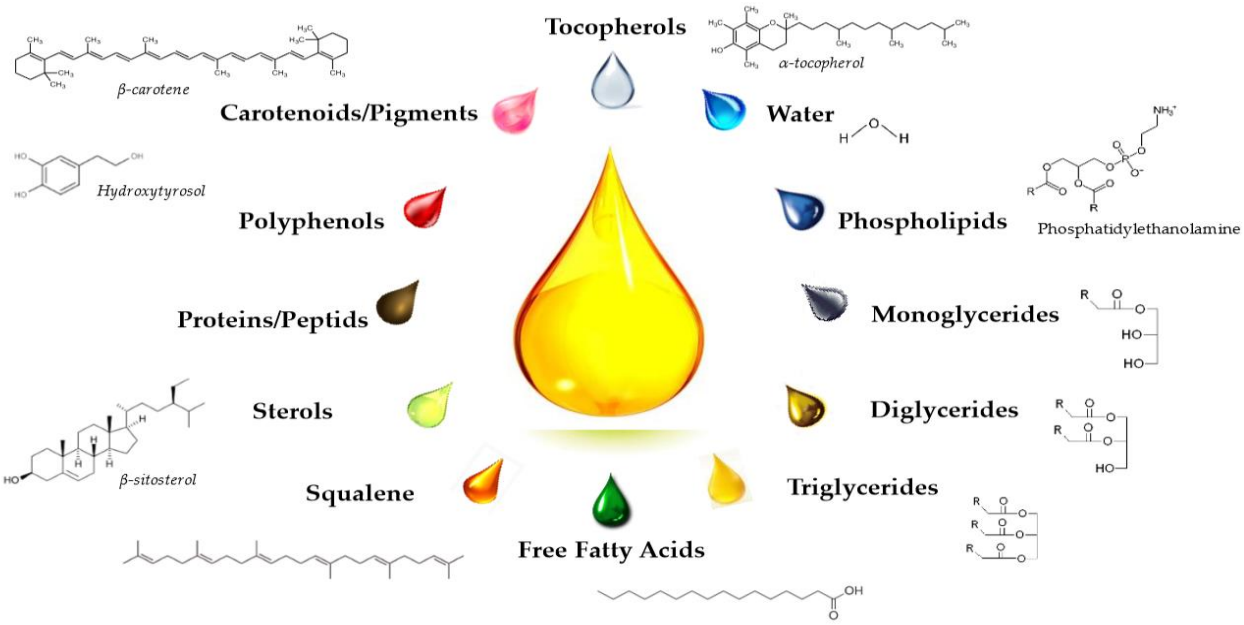


Figure 9: Minor components in soya oils **(Yara-Varón *et al.*, 2017)**

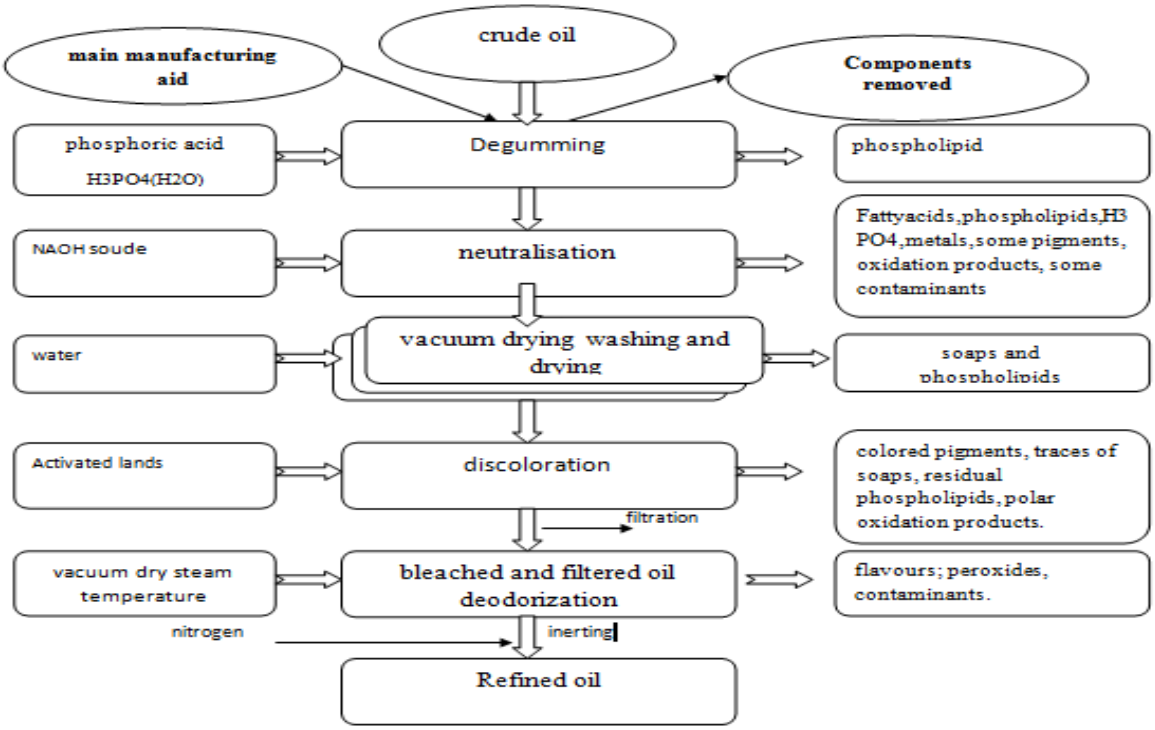


Figure 10: Steps in chemical refining **(Evrard *et al.*, 2007)**

I.6 Enrichment of vegetable oils

a) Solid liquid extraction

In this type of enrichment, a quantity of powder of the vegetable matter (solid) is partially dissolved in the oil. The passage of the active substances in the oily phase is therefore a function of the solubility of each compound (**Han *et al*., 2007).**

b) Liquid liquid extraction

It consists to make an oil in contact with an alcoholic solution of phenols, this is how these molecules are transferred to the oily phase as a function of their distribution factor and the alcohol phase is removed by centrifugation **(Han *et al*., 2007).**

c) Combination of the two methods

In this method, it is only after extraction of the polyphenols from the matrix that they are added to the oil and the whole is mixed. The separation of the two phases obtained is carried out under vacuum by elimination of alcohol **(Han *et al*., 2007).**

In recent years, different studies have used edible oil as a solvent for extracting substances of interest from different plant matrices. This extraction is favored and accelerated by the application of ultrasound **(Achat *et al*., 2012; Li *et al*., 2013; Penalvo *et al*., 2016).**

- Table below present examples of some works of enrichment of edible oils with antioxidant **(Tab. IV).**

Table IV: Enrichments methods with food aroma

Matrix	Analyte	Operating, condition and remarks	Reference
Vanillapods	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)**
Feuilles de basilic	Essentiel oil (Linalool; Eugénol)	Direct enrichissement (solide -liquide) Conventionnel méthode: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 aurantiumpeel	Essential oil	Flavoured oil (8:3 a, 1 h, 20◦C, 100 rpm) showed the highest total volatiles with unchanged fatty acid composition.	**(Karoui*et al*., 2010)**
Crawfishwaste	Astaxanthin	Maximal pigment extraction and oil recovery was obtained from a 1:1 a ratio of oil to crawfish waste	**(Chen and Meyers, 1982)**
Air-dried, powdered Lamiaceaeplants	Antioxidants	Enriched oils contain 0.1~0.5% of organic solvent extracts, in which Saturejahortensis L. ethanol extracts performed the best in oil stabilization	**(Marinova and Yanishlieva, 1997).**

Bitter orange peels*(Citrus aurantium L.)*	Monoterpenehydrocarbons(limonene)	500 ml as lipid matrix. Orange peels homogenized in the oil using incubator shaker (Excella E-24/24R Benchtop, New Brunswick Scientific CO., INC.). Incubation period (1 h, 2 h and 3 h) and peel quantity (5 g, 10 g and 15 g) were optimizedwhile oil volume (40 ml), incubator temperature (20 ◦C) andShaking speed (100 rpm) .Retention of volatile compounds by orange peels.	**(Karo *et al.*, 2010).**
Thyme dried flowers (Thymus capitatus)	Antioxidants	Antioxidant activities of the thyme-enriched oil were mainly due to the presence of phenolic compounds such as thymol and hydrocarbons such as γ-terpinene and p-cymene. The thyme-enriched oil could be considered as a new and natural source of antioxidant.	**(Karoui*et al*., 2016)**

MATERIALS AND METHODS

II. Materials and method

II.1 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 ($FeCl_3$, 6H2O). Potassium Iodure, Methanol, Cyclohexane, Ethanol, potassium ferricyanide [K3Fe (CN) 6], Chloroforme, potassium persulfate, ABTS, were supplied from Biochem-chemopharma (UK) however sodium dihydrogen phosphate from VWR (France).

II.2 Materials

Uv-vis spectrophotometer (Shimatzu, Japan), Microwave23L (NN-S674MF. Maxi power, China), analytical balance, precision balance (Radwag, UK), drying oven (Nuve, Turky), heating plate, vortex mixer, multiple heating Magnetic stirrer (VELP, Italy), Water bath (Memmert, Germany), pH meter (Hanna, France), Ultrasonic cleaner (Bransonic, USA), Centrifuge (hettich, Germany), Testo polar Compounds analyzer (Germany), Rancimat model 743(Metrohm, Swetzerland), industrial drying oven (TSENG,China), industrial extractor (Steam, Local).

II.3 Plant material

The plant was collected from Aokas (Bejaia) in April, 11th2019. The geographical position of this region is: 36°38'05.2"N ET 5°13'46.4"S. After identification, the harvested plant materials were washed with running tap water to remove surface contaminants. The samples were dried in an industrial drying oven at 30 °C until constant weight.

II.4 Evaluation of moisture content

Thermal drying method was used in the determination of moisture content of the sample.10 g of sample were placed in a drying oven, to dryness at 103 ± 2 ° C, until constant weight. The moisture content (MC) was calculated by the following formula **(Doymaz *et al.*, 2004):** Where W_0 correspond to the loss in weight (g) on drying and W_i correspond to the initial weight of sample.

$$MC\% = \frac{Wi - Wo}{Wi} X100$$

II.5 Vegetable oil

The soybean oil was supplied from research development laboratory (R&D) of Cevital Group (Bejaia).

II.6 Extraction procedures of essential oil

A preliminary study was performed in order to select the method type for the rest of investigation. In order to have the best yield extraction of rosemary EO, we have tested different techniques: hydro-distillation, microwave with hydro-distillation, ultrasound-microwave assisted hydro distillation and hydro-diffusion. All these operations were done in triplicate.

a) Microwave assisted hydro-distillation (MAHD)

MHD has been performed using a domestic microwave oven, 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 (the latter linearly adjustable from 200 to1000 W). The oven was modified in order to condensate in to the sample the vapors generated during extraction giving a constant sample volume**(Dahmoune *et al*., 2013).** For extraction of EO, 20 g of accurately weighed rosemary sample was heated using a fixed power of 700 W for 20 min, using 200 mL of water**(Liu *et al*., 2011).**

b) Hydro-distillation (HD)

300 g of sample and 3l of water was extracted with hydro-distillation for three3 hours.**(Hendel, Larous et al. 2016)**

c) Ultrasound and MAHD (US-MAHD)

20g of rosemary aerial part, in 400ml of water, was submitted to sonication treatment then extracted using microwave (20 min, 700W)

d) Steam distillation (SD)

10kg of dried leaves and flowers was put in an industrial extractor working steam distillation for 03 hours.

- The extraction yield of rosemary EO was obtained according to (**Liu *et al*., 2011)** , and calculated following the equation:$x = \frac{We}{Wp}$x100Where:

We: is the weight of the extract (essential oil),

Wp: weight of the plant used for the extraction.

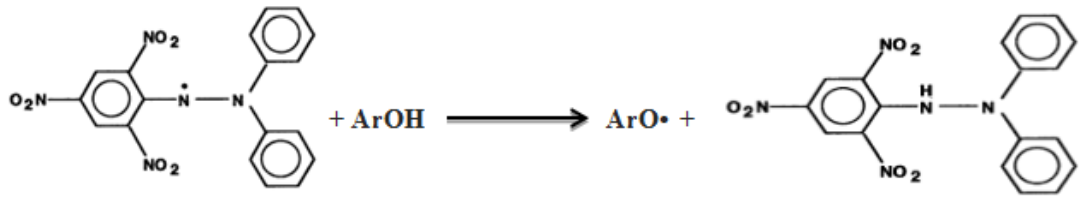
II.7 Antioxidant activities

II.7.1.DPPH° assay

The radical-scavenging activity of samples (EO and oils), was evaluated by the DPPH• assay. DPPH° is a stable highly colored free radical that can abstract labile hydrogen atoms from antioxidant (ArOH) with concomitant formation of a colorless hydrazine (DPPH-H), according to reaction in figure 2**(Molyneux, 2004)**. The free radical-scavenging activity (RSA) of an extract can be expressed as the percentage of DPPH reduced by a given amount of extract. The RSA was measured, following (**Hussain *et al.*, 2010)**method. 12.5 µL of each extract was added to 1.25 ml of DPPH solution (4.10^{-4} M) and the mixture was left in the dark at room temperature for 30 min. The quantity (mg) of dry extract per mL of reaction medium necessary to decrease the initial DPPH radical concentration by 50% (IC_{50}) was determined using an exponential curve. The total RSA of each extract was expressed as the percentage of DPPH reduced and was calculated by the following equation:

$$\text{RSA (\%)} = \frac{Ac - As}{AC} \times 100$$

A_c, absorbance of DPPH solution without any antioxidant; As , absorbance of DPPH solution after reaction with the extract. All experiments were performed in triplicate.



Diphenylpicrylhydrazyl (Free radical) **Diphenylpicrylhydrazine (Nonradical)**

Figure11: DPPH° radical reduction **(Molyneux, 2004)**.

- Ascorbic acid was used as standard antioxidant. All tests were carried out in triplicate.

II.7.2.Reducing power

The reducing power was determined according to the method of**(kadri*et al.*, 2011)**500µl of each sample at different final concentrations was dissolved in ethanol and mixed with 1.25 ml of reagent of 0.2 M phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferracyanide. The mixture was incubated 30 min at 50°C, followed by addition of 1.25 ml of 10% (w/v) trichloroacetic acid. The mixture was then centrifuged at 1500 g for 10 min.

Finally, 1.25 ml of the supernatant solution was mixed with 1.25 ml of distilled water and 250 µl of 0.1% (w/v) ferric chloride. After 10 min, the absorbance was measured at 700 nm). A higher absorbance indicates a higher reducing power. RC_{50} (mg ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid was used as standard antioxidant. All tests were carried out in triplicate.

II.7.3.ABTS free radical–scavenging assay

ABTS radical scavenging activities of the samples were assessed by the method of (**Re *et al.*, 1999).** Briefly, ABTS radical solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. After addition of 1.0 ml diluted ABTS radical solution to 10 µl of the sample, absorbance reading was taken 5 min after initial mixing. Ascorbic acid was used as reference. The results were expressed as the percentage of ABTS reduced and was calculated by the following equation:

$$\%= [1-(A_{sample}/A_{ABTS}solution_{)}] \times 100$$

A_{sample}: absorbance of extract solution; A_{ABTS}:absorbance of ABTS radical. All assays were made in triplicate.

II.8 Enrichment of soybean oil

II.8.1Preliminarystudy

A preliminary study was performed in order:

- To select the better method for enrichment, using conventional maceration (CM) and ultrasound assisted maceration (UAM).
- Todetermineoptimalliquid–liquidratiofortherestofinvestigation.Amountsofessential oilwere subjected to enrichment of soya oil: 5to150mgEO in 100gofedible oil under sonication, during1min to 15min. Experiments were followed using DPPH° test.

a) Ultrasound assisted maceration (UAM)

An ultrasonic apparatus (Tierratech Ultrasonic cleaner bath: Model: LT-80 PRO) was used for enrichment of oils, with working frequency fixed at 100 w. Different amount of EO were added to different volume of oils, as solvent into the ultrasonic device during different time.

b) Conventional maceration (CM)

Different amount of rosemary EO were added to oils, under stirring, during 10 mn for preliminary study. Conventional, made for comparison.

❖ Experimental design: Theory and application

The notion of the experimental designs was developed in the last decade in biology. Experimental design is a method of optimization trials of scientific research or industrial studies and provides the maximum amount of information with the minimum of experience. It also makes it possible to obtain the best possible precision on the modeling of the results. This method is based on strict mathematical rules; it requires a rigorous procedure on the part of the experimenter **(Achat, 2013).** They are applicable to many disciplines in all industries from the moment we look for the link between a quantity of interest, y, and variables, xi. We must therefore think of the plans of experiments if we are interested in a function of the type: y = f (x1, x2, x3, .. xi) **(Goupy, 2006).**

❖ Experimental Design (Box-Behnken Design)

Box-Behnken Design (BBD) is a second-degree multivariate model, it is easy to implement and possess sequentially property. Its main characteristics are as follow:

• Requires an experiment number N = 2k (k-1) + Cp ... (1), where k is the number of factors and Cp is the number of central points.

• All factor levels must be adjusted only at three levels (-1, 0, +1) with regular intervals. **(Bezerra *et al*,. 2008)**.

In our study, we applied a second-level Box-Behnken plan to evaluate the combined effect of two independent variables: ratio, time, which are designated X1 (concentration on EO: ppm) and X2 (Time: min) respectively. The preliminary study that we carried out, allowed us to determine the low and high levels for the variables influencing the experiments, which are illustrated in the **Table V.**

Table V: Experiments matrix

Assay	X1	X2
1	25	2
2	50	2
3	75	2
4	25	6
5	50	6
6	50	6
7	50	6
8	75	6
9	25	10
10	50	10
11	75	10

Thus 11 experiments will be carried out in order to estimate the mathematical model of the response investigated. The methodology of the response surfaces will make it possible to model the answers studied in the form of a polynomial equation of the second degree.

❖ Statistical analysis

All experiments were conducted in triplicate and results are expressed as mean ±standard deviation (SD). Statistical analysis was performed by analysis of variance in the software JMP.7. Differences were considered to be significant at $p < 0.05$.

II.9 Oxidation acceleration test

This test is widely used to assess the quality of edible oils and fats by evaluating in a quick and simple way, the stability and durability of the products, this is done in a way fully automatic. The device used is the "Rancimat", the specification of the induction time in the test "**Rancimat**", expressed in hours (h), corresponds to the time during which the fat has resisted oxidative stress. The principle of the test consists in premature ageing of fat by thermal decomposition at a specific temperature under intensive air bubbling. The degradation products of this extensive oxidation are carried away by an air current and collected in a measuring cell filled with distilled water **(Rahmani, 2007)**.

An **ISO standard: 6886**Rancimat method was used. A 3.0-g portion of each test sample was loaded into the reaction vessel. Measurements of three different samples were conducted in one batch. The air supply was maintained at 20 L/h and the heating temperature at 98 ºC throughout the experiment. Induction time for the test samples was

determined by measuring the elapsed time from the beginning to the moment when a sudden change of conductivity occurred. The control was soybean oil with no additives.

II.10. Heating conditions

Effect of heating conditions was studied by the determination of Total Polar Materials (TPM). Four hundred grams of oil (Soya, Soya-EO) was heated under domestic frying conditions, i.e. 180 ± 5 C during four hours. The temperature was monitored by a thermocouple (ATC-300) inserted directly into the domestic deep-fat electric fryers. All samples were evaluated before the first heating sessions and at 1 h of heating until oil discard. The end of heating assays was determined by the value of TPM, max 25% in accordance to the French law (Article3-3 of decree N86-857 of 18/07/86). This maximal legal content of TPM in frying oils, including hydrolysis products (diglycerides, monoglycerides and free fatty acids) and a complex distribution of oxidation products encompassing polymers, all formed at temperature below 180 °C (French law N86-857). The TPM value is usually assessed in restaurants and the agro food industry by fast commercial tests (mostly based on colorimetric readings), which have proven to correlate well with values obtained by official standards **(Achat *et al*., 2012).**

II.11Acidity and acid number

These tests were determined using ISO 660/1996 method. The acidity expressed conventionally as a percentage of fatty acid predominant in the fatty body, for oleic soybean oil. The acid number is the number of mg of KOH or NaOH required neutralizing the free fatty acids contained in 1g of fat. The principle is to neutralize fatty acids with a base in the presence of phenol-phthalein as colored indicator.

$$RCOOH + NaOH \longrightarrow RCOONa + H2O$$

- Weigh 5g of oil, heat a volume of 50 ml of 95% ethyl alcohol. After mixing, add 1ml of phenol-phthalein, shake and titrate with NaOH solution until the pink turn persist 10 seconds. Acidity and acid number were obtained as follow

$$\text{Acidity} = \frac{NxVxGE}{10xP}$$

$$\text{Acid number} = \frac{NxVx56.1}{P}$$

With:N: NaOH normality; V: volume spent for titration; P: test sample; GE: gram equivalent of the fatty acid in which the acidity is expressed (282 for oleic acid).

II.12Peroxide value

The peroxide value was assessed following the **ISO 3960:2007**technique.It represents the quantity of product present in the sample, expressed in milliequivalents of active oxygen per kilogram, oxidizing potassium iodide under the described operating conditions**.** Weigh 5g of oil in a bottle add 12ml of chloroform and 18ml of glacial acetic acid and then 1ml of saturated potassium iodide solution. Immediately plug the bottle, shake it for one minute and leave it out of the light, for one minute at room temperature. Add 75ml of distilled water. With vigorous stirring and in the presence of a few drops of starch as a color indicator, titrate the iodinated product with sodium thiosulfate solution (0.01N). In parallel with the determination, carry out a blank test. Thus, the peroxide value of the sample, is obtained:

$$PV = \frac{T(V1-V0)}{m} x1000$$

With: V0: the volume in ml of the sodium thiosulphate solution used for the blank test;

V1: the volume in ml of the sodium thiosulphate solution used for the determination;

T: the normality of the sodium thiosulphate solution used;

m: the mass in grams of the test sample.

II.13Sensory evaluation

Evaluation of sensory properties of soya oils (with or without rosemary EO) was performed according to the method of (**Achat *et al.*, 2012).**The panel was constituted by eight tasters, selected from university expert staff. Panelists evaluated the color, taste, texture, and odor of each sample, using a numerical scale 1–5.Two samples were used in this assessment frit and mayonnaise, prepared with no and enriched soya oils.

RESULTS AND DISCUSSION

III.1 Moisture content

Drying of herbs has been found to be a very useful technique for increasing the amount of phenolic compounds and antioxidant capacity of the extracts (**Hossain *et al*., 2010).** The fresh plant material contains high water content. Moisture promotes enzymatic activities which lead to irreversible changes in antioxidants after plant material harvest, such as oxidation, and therefore polymerization or decomposition **(Veillet, 2010).** Inaddition, moisture also help the development of microorganisms and molds that cause massive and rapid degradations of vegetable matrix during storage **(Johns, 1999)**.

The drying efficiency was evaluated in terms of water loss; the value obtained is about 28,2±0,9% which is approximatively near than moisture content reported by (**Hossain *et al*., 2010)**(24,8±0,63%).

III.2 Extraction procedures of rosemary essential oil

Rosmarinus officinalis L. essential oil is usually isolated by hydro distillation, steam distillation, or extraction with organic solvents. Those techniques cause the loss of certain volatile compounds due to long extraction times and degradation of unsaturated or esterified compounds by thermal or hydrolytic effect. For example, monoterpenes may be susceptible to chemical changes under stream distillation conditions and even the conventional solvent extraction during removal of solvent by distillation. In addition, many of these methods are time-consuming and energy intensive **(Maria Lo Prest*et al*., 2004; Bousbia *et al*., 2009**).
However, in order to reduce the extraction time and improve the quality of essential oils, new extraction techniques have been developed such as microwave assisted extraction, solvent extraction under pressure, supercritical fluid extraction, and ultrasound-assisted extraction **(Kaufmann *et al*., 2002; Wang *et al*.,2006).** Microwave-assisted hydro distillation has been used for the extraction of laurel essential oils **(Kosar *et al*., 2014)**, lavender **(Issartier *et al*., 2013)**, thyme **(Golmakani and Rezaei. 2008)** and rosemary **(Okoh *et al*., 2010)**. Faced with all these innovative methods of extraction of essential oils, the choice of the most efficient method is relevant for a better optimization of time, yield, and cost of production. Thus hydro distillation (HD), steam distillation (SD), microwave assisted hydro distillation (MAHD) and ultrasound-microwave assisted hydro distillation (US-MAHD) were tested.

III.3 .Yield extraction of essential oil

The mean values of yield extraction of rosemary EO are shown in **figure 12**. The highest extraction yield was obtained with US-MAHD, whereas the extraction yield with the rest of methods extracted less EO.

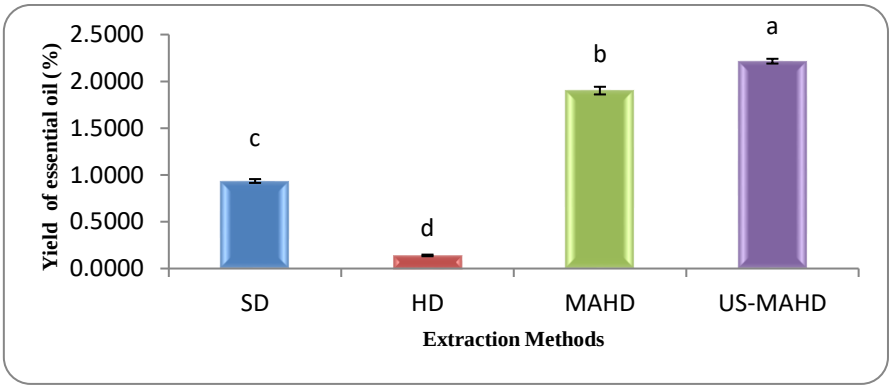


Figure 12: Comparaison between differents extraction methods of REO.

For all extraction techniques, SD, HD, MAHD, US-MAHD, the extraction temperature is equal to the boiling of the water at atmospheric pressure (100°C). To reach this temperature and to obtain the distillation of the first droplet of essential oil of rosemary, it is necessary to heat for 8min only with MAHD, USAHD against 45min for the VHD and HD. At 20-minutes extraction time by MAHD and USAHD give a better yield to that obtained after 180 min by means of VHD and HD.

Several studies have reported that the heat generated by the microwave heating involves a partial pressure gradient of volatile compounds and internal overheating leading to embrittlement or rupture of the cell walls more rapidly and more efficiently (**Bousbia *et al.*, 2009**). As a result, the kinetics of the extraction process of essential oils is accelerated, which explains the difference in time between the fourth extractions methods studied. This can be explained by the rate of heat transfer between the two extraction methods. MAHD and US-MAHD use three ways of heat transfer within the sample: irradiation, conduction, and convection, while the heat transfer by HD and SD can occur through conduction and convection only.

- The current results are not in accordance with those reported by **(Jaimand *et al.*, 2018)**: they found 1.30 % (HD), 0.54 % (SD) and 0.5 % (MAHD). The difference among the values of reported yield extraction of rosemary EO can be explained by the difference of the harvesting period (June-July) and the difference in origin of plant (Iran).

In our study, the US-MAHD was used for extraction of the rosemary's essential oil, due to its important extraction yield compared to the rest used methods, and the difference is significant.

III.3.1 Antioxidant assays of rosemary EO

III.3.2 DPPH° test

The DPPH radical is usually used as a substrate to evaluate the antioxidative action of antioxidants by determining the free radical-scavenging ability of various samples as demonstrated by**(Achat *et al.*,2012)**.**Figure 13** shows the DPPH° radical scavenging activity of different rosemary EO.

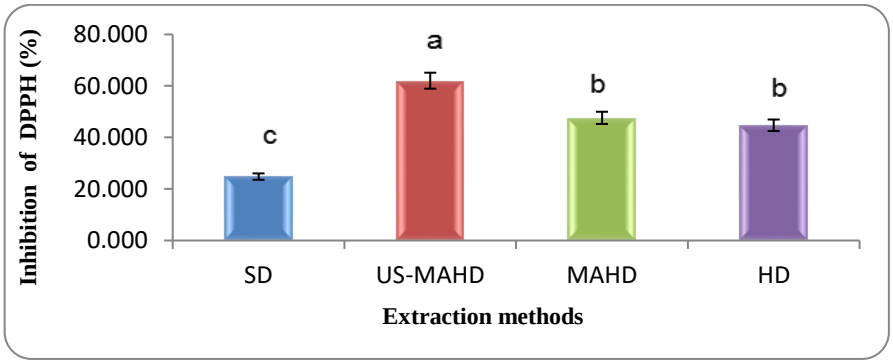


Figure 13: Antiradical scavenging activity (DPPH %) of different REO.

The essential oil extracted by US-MAHD exhibited the highest value of essential oil against DPPH capacity in comparison with SD, MHD, HD respectively. These results confirmed that USMHD is a better extraction method for essential oil than the others methods, but there is a non-significant difference activity between HD and MAHD, SD to other method.

III.3.3 ABTS test

US-MAHD provided a better technique that give a higher antiradical capacity of essential oil extracts**(Fig.14)**in comparison with SD;MAHD; HD of ABTS inhibition respectively as shown by DPPH test and there is a no significant difference between HD and MAHD.SD has a weak activity compared to other methods.

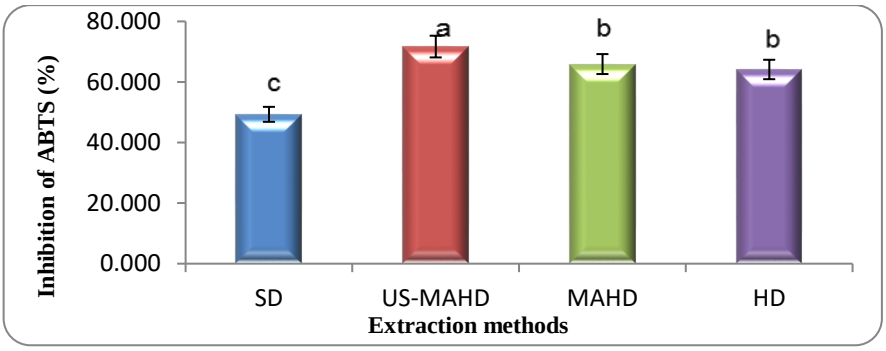


Figure 14: Antiradical scavenging activity (ABTS %) of different REO.

III.3.4 Reducing Power

All samples **(Fig.15),** showed their abilities to reduce Fe^{3+} to Fe^{2+}. The increase in the absorbance at 700 nm of the reaction mixture caused by the tested extracts is indicative of their increased reducing power.

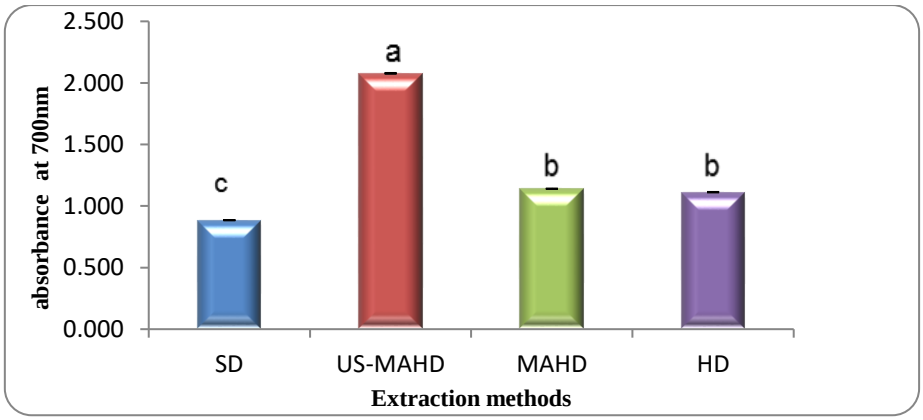


Figure 15: Absorbance (700nm) of reducing power of different REO.

After comparison, essential oil extracted by US-MAHD gives significatively a highest value of reducing power in comparison with SD, MAHD, HD: respectively. These results confirmed that US-MAHD method for is better than the others methods. However, SD has a weak activity compared to other methods and there is a non-significant difference between HD and MAHD.

III.4. Antioxidant activity of US-MAHD essential oil

The antioxidant capacities of US-MAHD essential oil are depicted in. The IC_{50} values (the concentration reducing 50% of radical) obtained for scavenging activities on DPPH°, ABTS radicals and reducing power were evaluated and compared to acid ascorbic.

The lower the IC_{50} value the greater the free radical-scavenging activity. Thus, Comparison of the antioxidant power of the investigated essential oil and those expressed by, showed that the oil possessed weaker antioxidant effects than ascorbic acid.

❖ Enrichment of soybean oil

III.4.1 Preliminary study

The effects of the variable of liquid–liquid ratio and the method of enrichment of soya oils with rosemary EO (CM and UAM) were evaluated. Before the choice of the experimental design different parameters have to be taken in our study and confirmed by the antioxidant activity (DPPH°) and oxidative stability (Rancimat and TPM).

III .4.1.1 Effect of time

The effects of time on independent variable using two enrichment methods of oils (CM and UAM) were evaluated using DPPH° activity **(Fig.16).** The best antioxidant activity was shown by enriched soya oil using US for 10 min compared to CV method at the same time (10 min). US produce ultrasonic waves that attack the integrity of plant cellular walls. This resulted in increased permeability of cytoplasmic membranes and more solvent can enter into the plant cell while causing the release of more compounds into the solvent **(Achat *et al.*, 2012).** Since shorter extraction time is also favorable to reduce energy costs, the 2– 10s range was selected for the RSM trials, while 10 min was kept for the next single-factor trials.

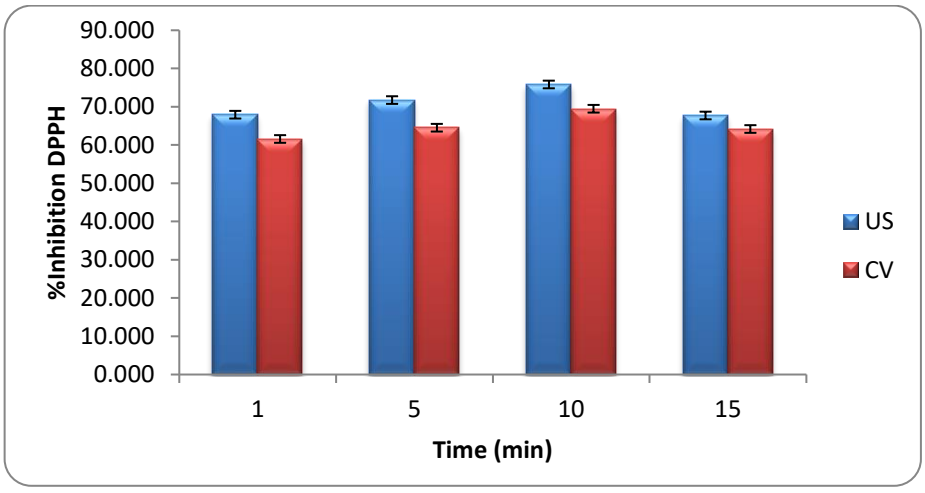


Figure 16: DPPH° activity of enriched soya oil with REO

III.4.1.1 Effect of Ratio

The effects of ratio: liquid-liquid variable in term of polar compounds, induction time using Rancimat and DPPH° assay were evaluated using different concentrations of rosemary

essential oil, to enrich soya oil **(Fig.17,18)**.The oils were exposed to heating at 180 ± 2°C for 4h.

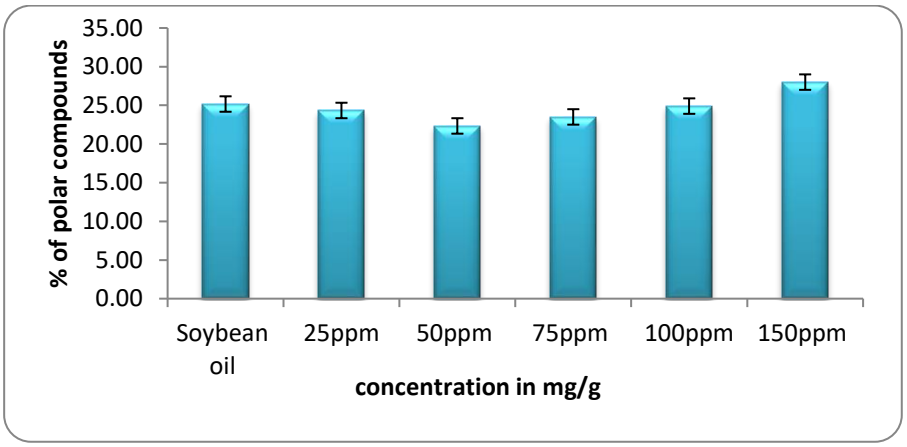


Figure 17: Total polar compound (%) of different ratio of REO in soya oil

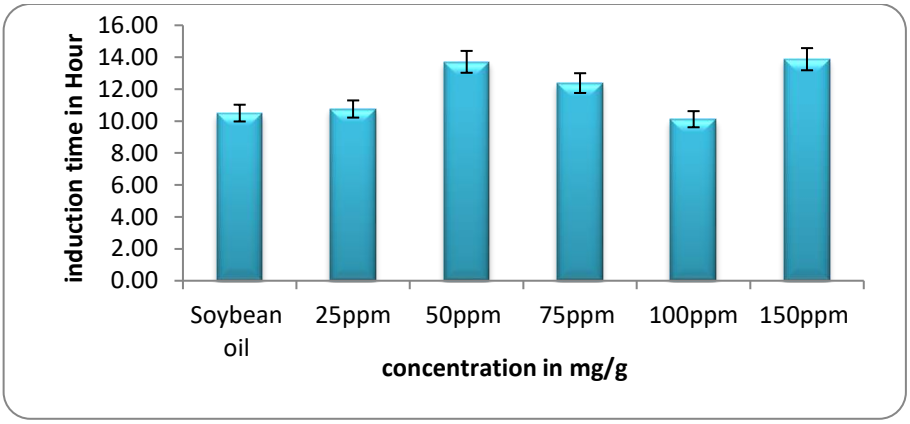


Figure 18: Induction time (hours) of different ratio of REO in soya oil

The concentration of 50ppm of EO shows a lower polar compound, and a higher induction time in comparison with other concentrations as demonstrated in figures above. Several authors revealed **(Réblová *et al.*, 1999; Sabrina and Neuza, 2012)** that the lower value of polar compounds and higher induction time lead to the best quality of oil.

- As demonstrated previously, another way to confirm the quality of our treated oils and confirm the concentration to use in experimental design, the DPPH radical scavenging activity was assessed **(Fig.19).**The results of 50 ppm EO, exhibit the highest activity compared to other concentrations.

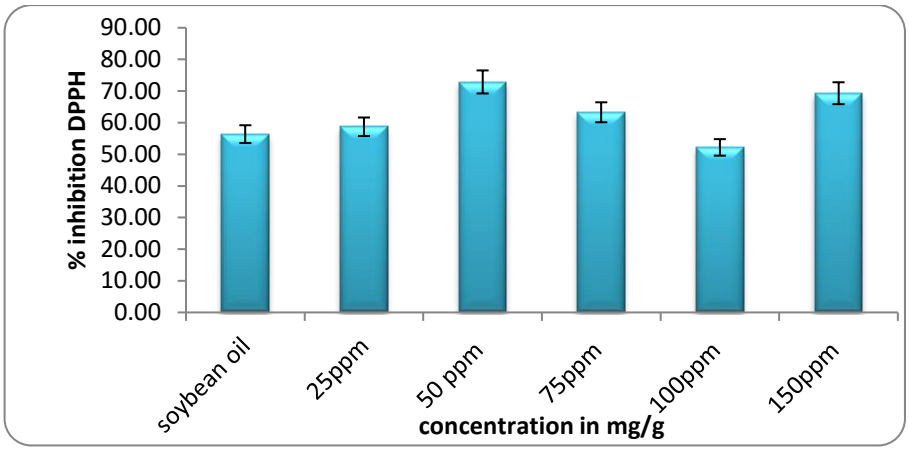


Figure 19: Antiradical scavenging activity of different ratio of REO in soya oil

III.5 Optimization of enrichment conditions

The experimental designs are very used in industrial studies in research and development; they allow a good understanding of the phenomena involved in the design of a new product and to apprehend a variable response quickly **(Achat *et al*., 2012).**

❖ Experimental Design

Eleven experimental points run randomly according to the UAE experiment planning, the coded values of independent variables and responses obtained in multivariate study for each experiment are shown in **Table VI.**

Table VI: Fully coded Box-benken design and responses obtained.

Assay	X1- Concentration (ppm)	X2-Time (min)	Experimental DPPH activity (Inhibition %)	PredictedDPPH activity (Inhibition %)
1	-1	-1	56,66	56,44
2	0	-1	52,91	54,82
3	+1	-1	47,13	45,43
4	-1	0	52,31	51,46
5	0	0	57,07	55,61
6	0	0	53,32	55,61
7	0	0	57,74	55,61
8	+1	0	49,87	51,98
9	-1	+1	57,56	58,62
10	0	+1	69,18	68,53
11	+1	+1	71,09	70,67

III.5.1 Modeling and fitting the model by RSM

The aim of UAE experiments was to find the relationship between independent variables and response, the mathematical model relates the response to the factors that influence it. In order to have a good answer several conditions must be verified. The regression model for extracts was given (discounting the non-significant parameters (p>0.05), in:

$$\mathbf{Y(DPPH)=55.61+ 6.85X_2- 3.89X_1^2+ 6.06X_2^2+ 5.76X_1X_2}$$

- The adjustment models obtained for the different responses are summarized in the figure below **:(figure 20)**

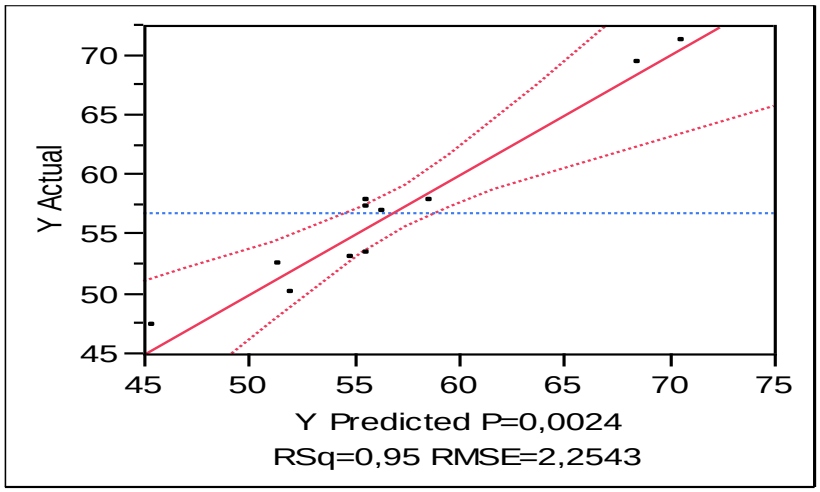


Figure 20: Predicted model

As can be seen, a good quality of fit is obtained for DPPH with an R^2 of 95%.This indicates a good representation and explanation of the variability of responses by the proposed model **(Li et *al.*, 2013).**Furthermore the value of the adjusted coefficient of determination is high (**R^2 adjusted 90%)** and the Lack of fit values are greater than 0.05 **(Tab.VII**,) which is evidence for the validity of this plan and seems to be sufficient for the experimental results to a level of confidence 95% **(Achat *et al.*, 2012).** Indeed**,** according to **(Le Man *et al.*, 2010)**, a model is considered adequate when R^2> 0.75. In addition, the ANOVA analysis shows that the polynomial model is highly significant with *P*-value <0, 05 and the lack of adjustment is not significant (P> 0.05), we compare it to pure error, which asserts that the model is adequate to express the rate of DPPH according to the two parameters to be optimized (concentration and time).

The results of the estimated parameters and the value of the predicted values given by the JMP software **Tinsson (2010)** are completed in the (**Table VII)**. The results presented that

the time of exposition in UAE(X_2) have a significant influence on the rate of DPPH, with a probability P = 0.0007.The concentration factor (X_1) has no significant effect on the rate of DPPH with a P = 0.7889.In fact, the time have an influence on the enrichment of the soybean oils by the essential oil of *Rosemarinus officinalis.* Our results obtained from the Box-Behnken model support these claims and are consistent with the works of **(Stavros and Vassilis2003; Tug˘ba and Medeni, 2013).**

Table VII: ANOVA for DPPH activity obtained in the BBD for EO

Parameter	DF	Sum of squares	F-value	Prob> F
Model Intercept (DPPH)	5	522,54994	20,5659	0,0024*
Linear				
X1	1	0,40560	0,0798	0,7889
X2	1	281,94615	55,4826	0,0007*
Quadratic				
$X1^2$	1	38,35540	7,5477	0,0404*
$X2^2$	1	93,15436	18,3313	0,0079*
Interaction				
X1X2	1	132,94090	26,1607	0,0037*
Lack Of Fit	3	14,059244	0,8259	0,5884
Pure Error	2	11,349267		
Total Error	5	25,408511		
R2			0.95	
R2 Adjusted			0.90	
Corr.Total	10	547,95845		

The quadratic effect influence very significantly the enrichment of soybean oil with a probability *P = 0,0404*and *P=0, 0079* respectively. These results reveal the importance of time and concentration for the enrichment of soybean oil, which can be seen in **(Table VII)**where there is a very significant effect of interaction between variables with a probability *P = 0.0037.*

From the observations made during the study of the response surfaces **(Fig. 22)**, the efficiency of soybean oil enrichment in terms of essential oil concentration increases by increasing of concentration and exposition time to the US.

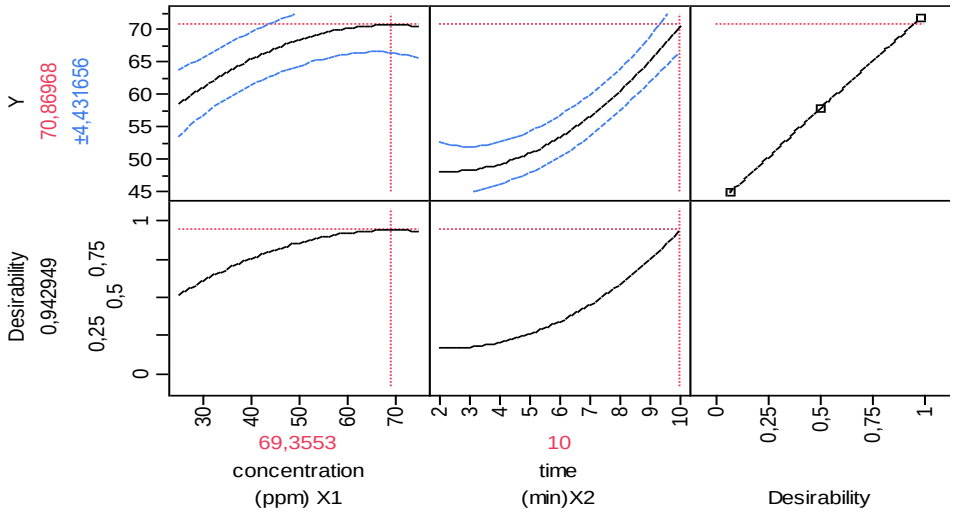


Figure 21: Optimal conditions predicted of enriched oil.

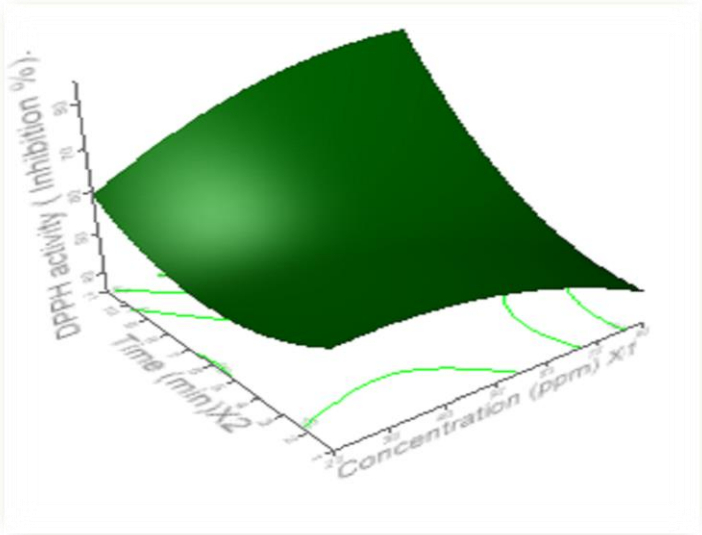


Figure 22: Response surface analysis for the DPPH° activity; from soybean oil with respect to sonication time and concentration.

As a result, the optimum conditions at most of these two criteria, namely 69,35ppm and 10 min of sonication time. Under these conditions, the experimental value of DPPH is 73±0,02%, which is reasonably close to the predicted value 70,86± 4,31%.In the light of the results obtained, we can conclude that the modeling of the response surface can be effectively applied for the enrichment of soybean oil by the essential oil of *Rosmarinus officinalis.*

- **Comparison of UAM and CM :**

Selection of an enrichment method would mainly depend on the advantages and disadvantages of the processes such as extraction yield, complexity, production cost, environmental friendliness and safety **(Achat *et al*., 2012).** A comparison was carried out between UAM and conventional

maceration method without application of ultrasound (CM), for the enrichment of soya oil with rosemary essential oil using optimal conditions (**Fig. 23, 24 et 25**). It is observed that the best antioxidant capacity (DPPH°) and the highest time of polar materials were assigned to UAM.

- In the light of the results of the anti-radical test of the oils (crude and enriched). It was indicated that the ability to scavenge the radical DPPH° by the enriched soybean oil is much greater (73, 34 ± 0.5%) US method and (70, 28±1, 15) CM method, than the crude soybean oil (58, 67 ± 0.4%).Results are in agreement with the works obtained by (**sabrina and neuzajorge, 2012).**

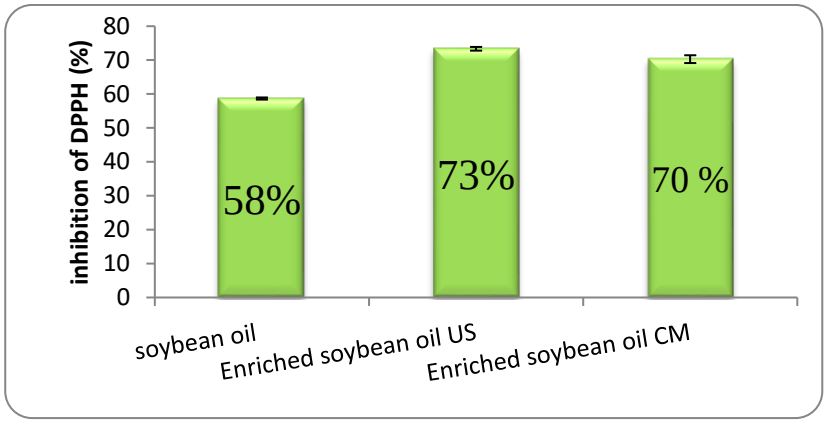


Figure 23: Antiradical scavenging activity (DPPH %) of soya oil and enriched soya oils

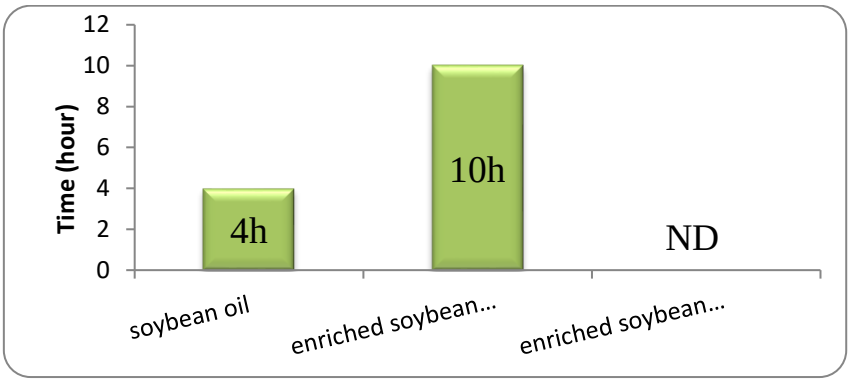


Figure 24: Heating effect on polar compounds of soya oil and enriched soya oils.

- Antioxidant activities were assessed using total polar compounds and oxidative stability in the samples collected after 0h to 10h**(Sabrina and Neuza, 2012).**The higher amount of polar compounds in oil, the worse its quality is (**Gharachorloo *et al*., 2010).** The concentration of products that formed as a result of oxidative alteration of the oil increased as a function of temperature, limiting the usefulness of the oil. In many countries, an upper limit of 25% total polar compounds has been established **(Angelo and Jorge, 2008)**. In our study, only treatments without rosemary's extract (SO) have values above this limit after 4 h of heating, and using the CM the polar compounds was not identified by the OPTIFRY instrument. These

results reinforce the strong antioxidant activity of rosemary extract **(Sabrina and Neuza Jorge, 2012).**

❖ Oxidation acceleration test

The Rancimat method may also provide some other useful information regarding oxidative stability of edible fats and oils and fat-containing foods **(Reza Farhoosh, 2007).**

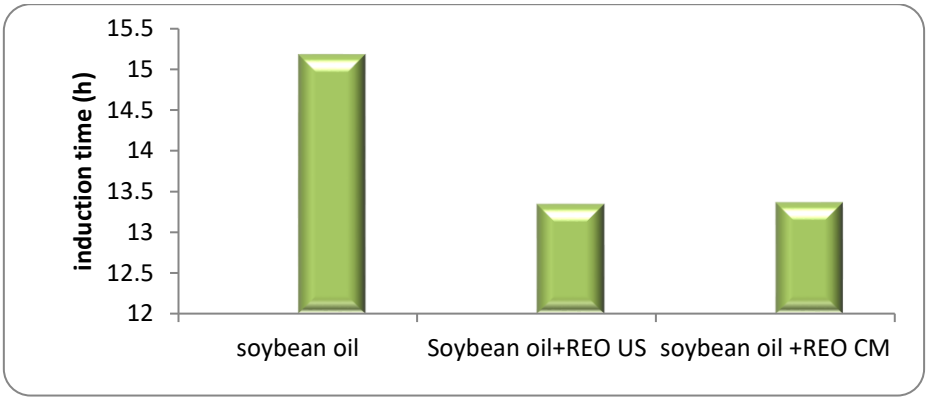


Figure 25: Induction time with Rancimat of soya oil and enriched soya oils

According the results obtained by Rancimat method **(Fig.25**), there is a negative influence of the REO on the induction time of the soybean oil. These results are not agreement with the work of **(S.P.J. NamalSenanayake, 2018).**

III.6. Sensory analysis

III.6.1 Mayonnaise

Two mayonnaise samples were used, three traditional formulations and flavored (A, B, C) are prepared. The samples were prepared at 3BS laboratory, they were then kept refrigerated (approximately 6C) until use (**M.J. SANTA CRUZ, 2001).**

- **Sensory profile**

The profile was measured by 8 member panel that had been screened and had received general sensory training. Each assessor had a minimum of 40 h experience in discrimination and descriptive analysis of different food products. Laboratory had individual booths with artificial illumination, temperature control and air circulation **(M.J. SANTA CRUZ, 2001).**

- **Product characterization**

The **figure26** present the characteristic ordered from the one having the highest discriminant power to the one having the lowest discriminant power on the prepared mayonnaise. As reported the color has the lowest discriminant power followed by the consistence the taste and the aroma at the last.

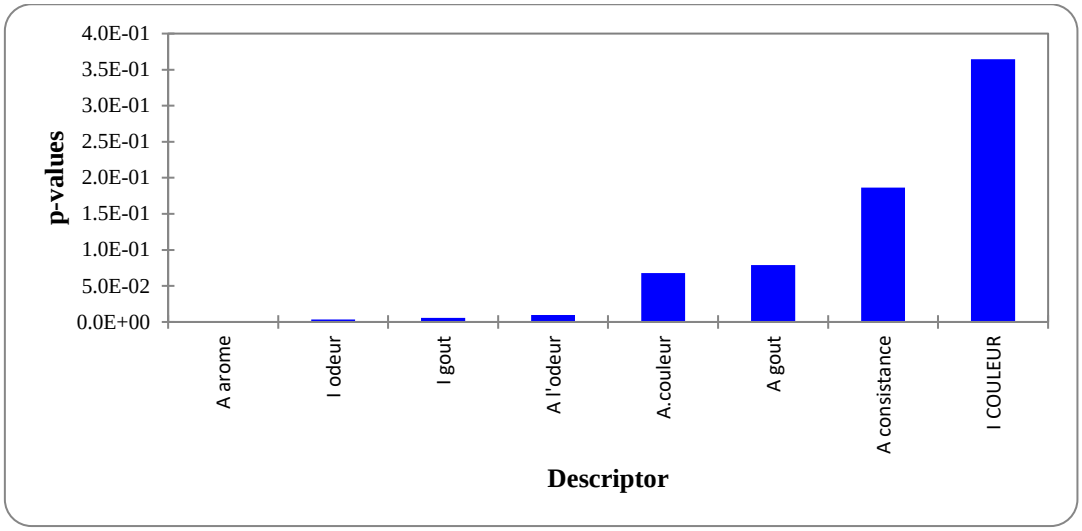


Figure 26: Discriminating power by descriptor.

- **Model coefficients**

General Procrustes analysis **(Arnold and Williams, 1986)** was used to monitor assessor's performance by analyzing their residuals and their relative position on the principal coordinate analysis plot. Analysis of variance (ANOVA) was performed on each sensory descriptor, and means were compared using Fisher's least significant difference (LSD).

Blue: coefficients are significantly positive

White: coefficients are not significant

Red: coefficient is significantly negative

The analysis of each graph defines each sample and shown in **figure27**. The figures indicates that:

- ✓ Sample A had a bad color.
- ✓ Sample A had a good color.
- ✓ Sample B had a bad color but good aroma, taste, flavor and odor.

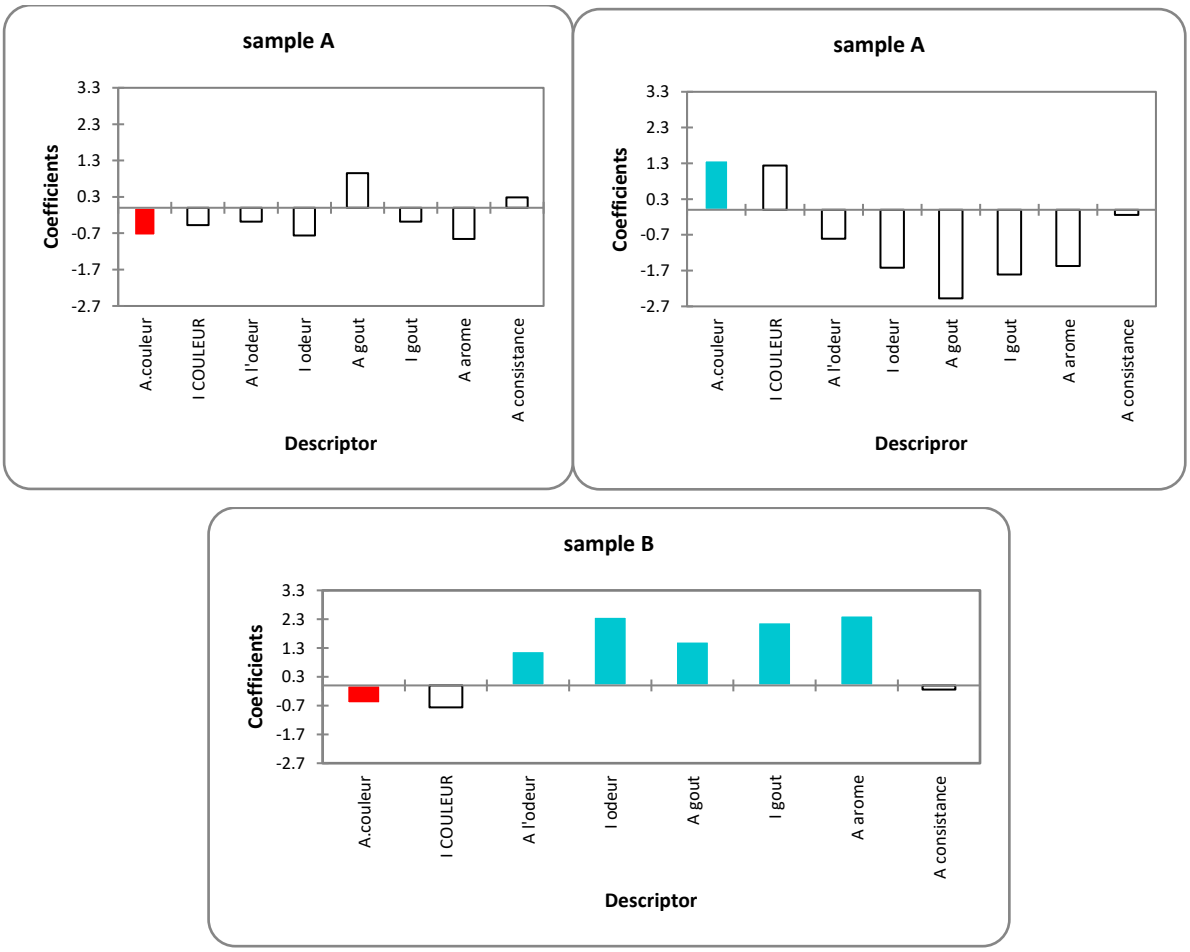


Figure 27: model coefficient of mayonnaise.

- **Adjusted product**

The purpose of this action is to define the adjusted product calculated from the model from each combination product descriptor **(Le and Husson, 2008)**. In this sensory evaluation results are shown in **(Table VIII).**

Table VIII : adjusted product for the mayonnaise

Samples	I taste	I odore	A odore	A taste	A aroma	A consistance	A.color	I CoLor
Sample B	4,7500	4,5000	3,0000	3,7500	4,6250	3,0000	3,8750	3,2500
Sample A	2,1786	1,3571	1,4286	3,1786	1,3393	3,4286	3,7321	3,5357
Sample A	0,7500	0,5000	1,0000	-0,2500	0,6250	3,0000	5,8750	5,2500

This table brings out the averages when passing the various products and feature. It is therefore seen in blue that are significantly larger than the overall average and red one are significantly smaller than the overall average. Mixture B had a good taste, odor and aroma opposed to sample A.

- **Preference**

The**figure28**represent the histograms of preference of each product by the jury. As it can be seen the sample B mayonnaise with enriched soy bean oil with rosemary's essential oil was more appreciated than the sample A which is the mayonnaise with only soybean oil.

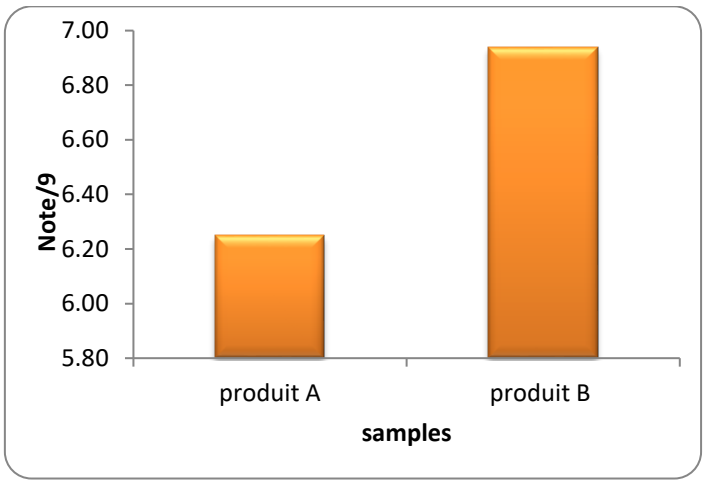


Figure 28: preference assigned to each product by subject

III.6.2. Potatoes fries

The preparation was effected with the same oils, place and conditions of analyzing were done for the potatoes fries, and samples A, B were prepared.

- **Product characterization**

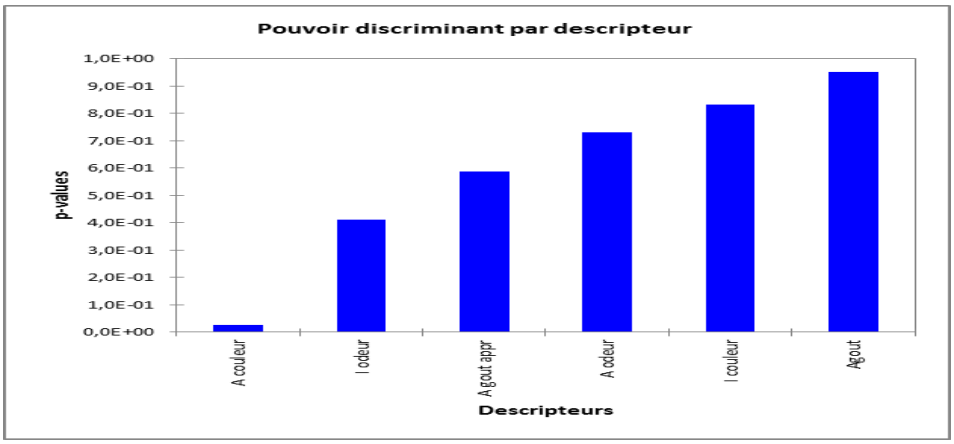


Figure 29: discriminator power for the potatoes fries

The **figure29**present the characteristic ordered from the one having the highest discriminant power to the one having the lowest discriminant power on the prepared potatoes fries. As reported the color has the lowest discriminant power followed by the taste, aroma and color at the last.

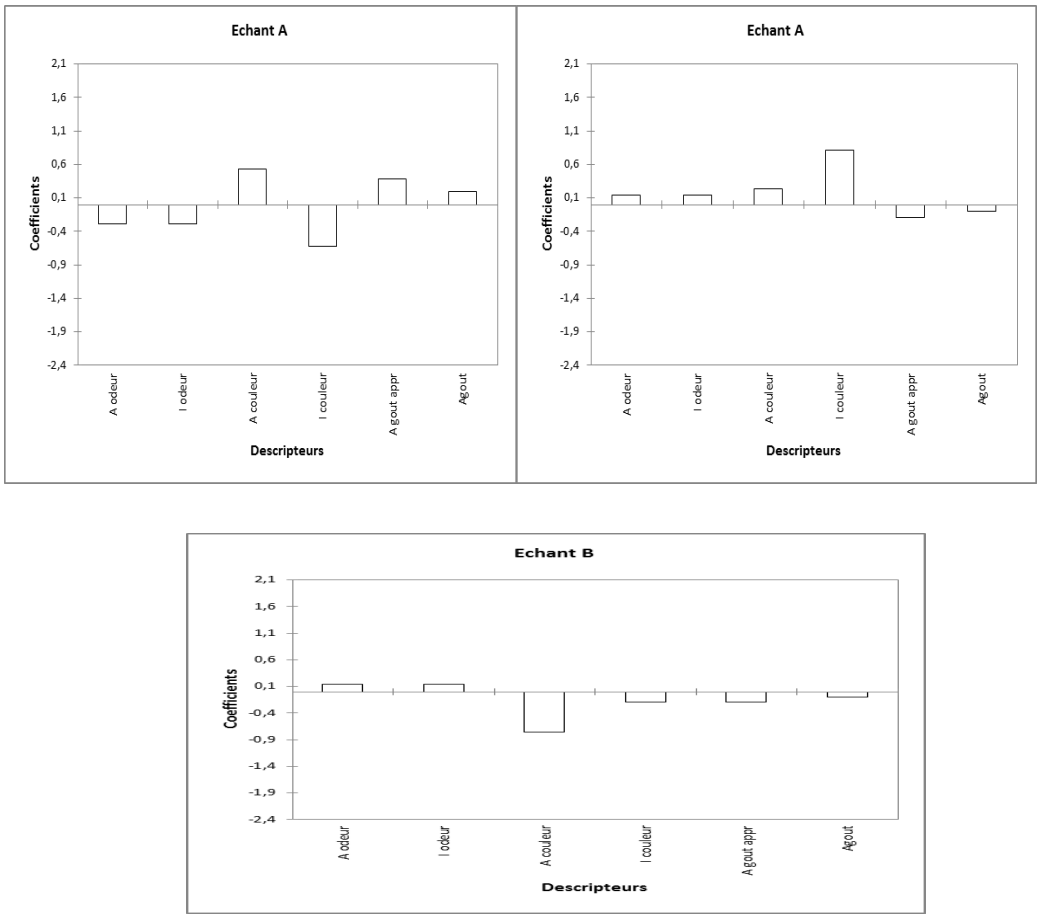


Figure 30: model coefficient of potatoes fries

The figure shows that there is no significant difference between the samples.

- **Preference**

The **figure31**represent the histograms of preference of each product by the jury. As it can be seen the sample B potatoes with enriched soy bean oil with rosemary's essential oil was not appreciated than the sample A which is the potatoes fries with only soybean oil.

Here the problem it was related to salt because the sample B more salt than A.

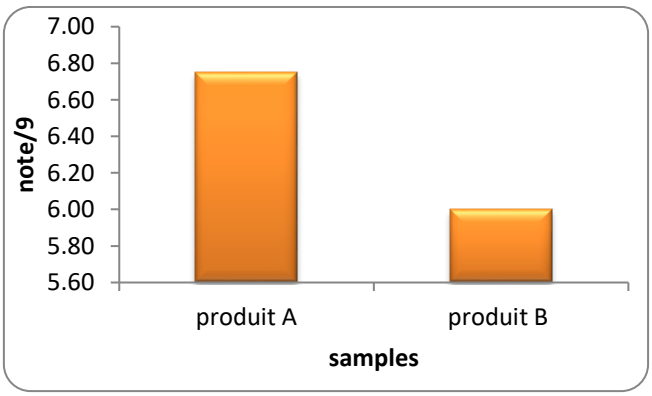


Figure 31: preference assigned to each product by subject

CONCLUSION

Rosemary is also known medicinally for its powerful antioxidant activity and antibacterial properties and as a chemo preventive agent. Today, essential oil of rosemary is widely used in the cosmetic industry producing various bathing essences, hair lotions and shampoos. Traditional methods used for extraction of essential oil were water distillation (WD) or steam distillation. Essential oils are well known to be thermally sensitive and vulnerable to chemical changes.

Our study is divided in three parts:

First, we compared between Water distillation (WD), steam distillation (SD) and microwave Assisted hydro distillation (MAHD) and a combination between MAHD and US (ultrasound), these techniques have been compared, for the extraction of essential oil from leaves of *Rosmarinus officinalis* L. This microwave extraction method offers important advantages over traditional water-distillation and steam distillation, shorter extraction times for 20 min against 3 h for hydro-distillation and steam distillation, better yields for MAHD and US-MAHD. Environmentally friendly lower cost and the possibility for a better reproduction of natural aroma of rosemary's essential oil than traditional distillation like Water distillation (WD), steam distillation (SD).

Secondly, we compared the antioxidant activity of the different oils obtained by different techniques of extraction, and the results were, for USMHD, better than other methods.

So the combination of ultrasound and micro waves shows synergies and need to be developed for the optimization of extraction and antioxidant activity of the essential oil.

Thirdly, the essential oil obtained by the USMHD was used in order to improve the stability of the soybean oil against oxidation and heating treatments.

The results of our study showed that the essential oil of rosemary improved the stability of the oil from 4h to 10h of heating at 180 C. The same oil was exposed to Rancimat method and shows that the induction time is improved from 10h for soybean oil to 13,88h for soybean oil enriched with 69,35ppm of essential oil, but in the other side soybean oil submitted to the Rancimat method without heating shows better oxidative stability compared to the soybean oil enriched with essential oil and these results have to be verified second time in order to know if really the enrichment of oil did not increase the stability of soybean oil unlike to the results found in the literature .

To support our study and to be sure that our results can be extrapolated on the consumer, two products based on frying oils were prepared in this case, the mayonnaise and fries, which were submitted to an expert tasting jury, and the results revealed that mayonnaise prepared

with oil enriched with essential oil of rosemary is appreciated, but for fries shows non-significant difference.

Finally we can say that the essential oil of the rosemary can be replaced as an alternative antioxidant in oil preservation.
As perspective, the replacement of the synthetic antioxidant by the naturel plant extract can be healthy for the human been and in the same time develop the world in general and our region in special.

BIBLIOGRAPHYCAL REFERENCES

A

Achat, D. L., L. Augusto, et al. (2012). "Drying-induced changes in phosphorus status of soils with contrasting soil organic matter contents–Implications for laboratory approaches." Geoderma**187**: 41-48.

Achat, S., V. Tomao, et al. (2012). "Direct enrichment of olive oil in oleuropein by ultrasound-assisted maceration at laboratory and pilot plant scale." Ultrasonics Sonochemistry **19**(4): 777-786.

Akhbari, M., S. Masoum, et al. (2018). "Optimization of microwave assisted extraction of essential oils from Iranian Rosmarinus officinalis L. using RSM Journal of food science and technology: 1-11.

Albu, S., E. Joyce, et al. (2004). "Potential for the use of ultrasound in the extraction of antioxidants from Rosmarinus officinalis for the food and pharmaceutical industry." Ultrasonics Sonochemistry **11**(3-4): 261-265.

Alizadeh, L., K. Nayebzadeh, et al. (2016). "A comparative study on the in vitro antioxidant activity of tocopherol and extracts from rosemary and Ferulago angulata on oil oxidation during deep frying of potato slices." Journal of food science and technology **53**(1): 611-620.

Andrade, M. A., R. Ribeiro-Santos, et al. (2018). "Characterization of rosemary and thyme extracts for incorporation into a whey protein based film." LWT**92**: 497-508.

Angelo, P. M. and N. Jorge (2008). "Antioxidant evaluation of coriander extract and ascorbyl palmitate in sunflower oil under thermoxidation." Journal of the American oil chemists' society **85**(11): 1045-1049.

Arnold, G. and A. Williams (1986). "use of generalised procrustes techniques in sensory analysis." Statistical procedures in food research/edited by JR Piggott.

Azmir, J., I. Zaidul, et al. (2013). "Techniques for extraction of bioactive compounds from plant materials: A review." Journal of food engineering**117**(4): 426-436.

B

Bakkali, F., S. Averbeck, et al. (2008). "Biological effects of essential oils–a review." Food and Chemical Toxicology**46**(2): 446-475.

Bousbia, N., M. A. Vian, et al. (2009). "Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity." Food Chemistry **114**(1): 355-362.

Burt, S. (2004). "Essential oils: their antibacterial properties and potential applications in foods—a review." International journal of food microbiology**94**(3): 223-253.

C

Casarotti, S. N. and N. Jorge (2014). "Antioxidant activity of rosemary extract in soybean oil under thermoxidation." Journal of Food Processing and Preservation **38**(1): 136-145.

Chahboun, N., A. Esmail, et al. (2014). "Extraction and study of the essential oil Rosmarinus officinalis cuellie in the region of Taza, Morocco." Pharma Chemica**6**: 367-372.

Chen, H. M. and S. P. Meyers (1982). "Extraction of astaxanthin plgment from crawfish waste using a soy oil process." Journal of Food Science**47**(3): 892-896.
Cossut, J., B. Defrenne, et al. (2002). "Les corps gras: Entre tradition et modernité."

D

Dessqualimapa (Gestion de la Qualité Nutritionnelle et Marketing des Produits Alimentaires), Institut Agro-Alimentaire de Lille Université des Sciences et Technologies de Lille, Institut d'Administration des Entreprises de Lille.

De Oliveira, J. R., S. E. A. Camargo, et al. (2019). "Rosmarinus officinalis L.(rosemary) as therapeutic and prophylactic agent." Journal of biomedical science **26**(1): 5.

E

Evrard, J., X. Pagès-Xatart-Pares, et al. (2007). "Procédés d'obtention et compositions nutritionnelles des huiles de tournesol, olive et colza." Cahiers de Nutrition et de Diététique**42**: 13-23.

F

Farhoosh, R. (2007). "The effect of operational parameters of the Rancimat method on the determination of the oxidative stability measures and shelf-life prediction of soybean oil." Journal of the American oil chemists' society **84**(3): 205-209.

Fazlali, A., S. Moradi, et al. (2015). "Studying of optimization condition of rosemary essence extraction with microwave assisted hydro-distillation method." Am. J. Essent. Oil Nat. Prod.**3**(1): 46-50.

Fellows, P. (2000). "Food Processing Technology Principles And Practice." Food Science and Technologie.

G

Gad, A. S. and A. F. Sayd (2015). "Antioxidant properties of rosemary and its potential uses as natural antioxidant in dairy products—A review." Food and Nutrition Sciences **6**(01): 179.

Gaussen, H. (1982). "Précis de botanique."

Gharachorloo, M., M. Ghavami, et al. (2010). "The effects of microwave frying on physicochemical properties of frying and sunflower oils." Journal of the American oil chemists' society **87**(4): 355-360.

Giugnolinini, L. (1985). "In Erbe Secondo Natura." Secondo Natura: Laboratorio Grafico, Vignate, Milano**4**.

Goetz, P. and K. Ghedira (2012). "Rosmarinus officinalis (Lamiaceae): Romarin." Phytothérapie anti-infectieuse: 341-347.

Golmakani, M.-T. and K. Rezaei (2008). "Comparison of microwave-assisted hydrodistillation withthe traditional hydrodistillation method in the extractionof essential oils from Thymus vulgaris L." Food Chemistry **109**(4): 925-930.

Gude, V. G., P. Patil, et al. (2013). "Microwave energy potential for biodiesel production." Sustainable Chemical Processes**1**(5): 1-31.Han, X., T. Shen, et al. (2007). "Dietary

polyphenols and their biological significance." International journal of molecular sciences **8**(9): 950-988.

H

Hendel, N., L. Larous, et al. (2016). "Antioxidant activity of rosemary (Rosmarinus officinalis L.) and its in vitro inhibitory effect on Penicillium digitatum." International Food Research Journal**23**(4): 1725.

Herrera, M. and M. L. De Castro (2004). "Ultrasound-assisted extraction for the analysis of phenolic compounds in strawberries." Analytical and bioanalytical chemistry**379**(7-8): 1106-1112.

Hossain, M., C. Barry-Ryan, et al. (2010). "Effect of drying method on the antioxidant capacity of six Lamiaceae herbs." Food Chemistry **123**(1): 85-91.

I

Ivanović, J., S. Đilas, et al. (2009). "Supercritical carbon dioxide extraction of antioxidants from rosemary (Rosmarinus officinalis L.) and sage (Salvia officinalis L.)." Journal of the Serbian Chemical Society**74**(7).

İnanç, T. and M. Maskan (2013). "Testing the antioxidant effect of essential oils and BHT on corn oil at frying temperatures: A response surface methodology." Journal of the American oil chemists' society **90**(12): 1845-1850.

J

Jadhav, D., B. Rekha, et al. (2009). "Extraction of vanillin from vanilla pods: A comparison study of conventional soxhlet and ultrasound assisted extraction." Journal of food engineering**93**(4): 421-426.

Jarboui, R., F. Sellami, et al. (2010). "Olive mill wastewater evaporation management using PCA method: case study of natural degradation in stabilization ponds (Sfax, Tunisia)." Journal of hazardous materials**176**(1-3): 992-1005.

K

Kabuba, J. and R. Huberts (2009). "Steam extraction of essential oils: investigation of process parameters." The Canadian Journal of chemical Engineering**87**(6): 915-920.

Karakaya, S., S. N. El, et al. (2014). "Microwave-assisted hydrodistillation of essential oil from rosemary." Journal of food science and technology**51**(6): 1056-1065.

Karoui, I. J., K. Msaada, et al. (2016). "Bioactive compounds and antioxidant activities of thyme-enriched refined corn oil." J. Agric. Sci. Technol**18**: 79-91.

Karoui, I. J., W. A. Wannes, et al. (2010). "Refined corn oil aromatization by Citrus aurantium peel essential oil." Industrial Crops and Products**32**(3): 202-207.

Kaufmann, B. and P. Christen (2002). "Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction." Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques **13**(2): 105-113.

Khalili, G., A. Mazloomifar, et al. (2017). "Supercritical Fluid Extraction as a Technique to Obtain Essential Oil from Rosmarinus officinalis L." Oriental Journal of Chemistry**33**(5): 2537-2541

Kosar, M., T. Özek, et al. (2005). "Comparison of microwave-assisted hydrodistillation and hydrodistillation methods for the analysis of volatile secondary metabolites." Pharmaceutical biology **43**(6): 491-495.

L

Lalas, S. and V. Dourtoglou (2003). "Use of rosemary extract in preventing oxidation during deep-fat frying of potato chips." Journal of the American oil chemists' society **80**(6): 579-583.

Le Man, H., S. Behera, et al. (2010). "Optimization of operational parameters for ethanol production from Korean food waste leachate." International Journal of Environmental Science & Technology **7**(1): 157-164.

Lê, S. and F. Husson (2008). "SensoMineR: A package for sensory data analysis." Journal of Sensory Studies **23**(1): 14-25.

Lo Presti, M., S. Ragusa, et al. (2005). "A comparison between different techniques for the isolation of rosemary essential oil." Journal of separation science **28**(3): 273-280.

Liu, T., X. Sui, et al. (2011). "Application of ionic liquids based microwave-assisted simultaneous extraction of carnosic acid, rosmarinic acid and essential oil from Rosmarinus officinalis." Journal of Chromatography A**1218**(47): 8480-8489.

M

Marinova, E. and N. Yanishlieva (1997). "Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil." Food Chemistry**58**(3): 245-248.

Martínez, C., M. a. J. Santa Cruz, et al. (2002). "Preference mapping of cracker type biscuits." Food Quality and Preference **13**(7-8): 535-544.

Mohamed, A. A., G. A. El-Emary, et al. (2010). "Influence of some citrus essential oils on cell viability, glutathione-S-transferase and lipid peroxidation in Ehrlich ascites carcinoma cells." Journal of American Science**6**(10): 820-826.

N

Nerio, L. S., J. Olivero-Verbel, et al. (2010). "Repellent activity of essential oils: a review." Bioresource technology**101**(1): 372-378.

O

Okoh, O., A. Sadimenko, et al. (2010). "Comparative evaluation of the antibacterial activities of the essential oils of Rosmarinus officinalis L. obtained by hydrodistillation and solvent free microwave extraction methods." Food Chemistry **120**(1): 308-312.

Orhan, I., S. Aslan, et al. (2008)."Inhibitory effect of Turkish Rosmarinus officinalis L. on acetylcholinesterase and butyrylcholinesterase enzymes." Food Chemistry**108**(2): 663-668.

P

Périno-Issartier, S., C. Ginies, et al. (2013). "A comparison of essential oils obtained from lavandin via different extraction processes: ultrasound, microwave, turbohydrodistillation, steam and hydrodistillation." Journal of Chromatography A **1305**: 41-47.

R

Rasolohery, C. A. (2007). Étude des variations de la teneur en isoflavones et de leur composition dans le germe et le cotylédon de la graine de soja [Glycine max (L.) Merrill].

RÉBLOVÁ, Z., J. KUDRNOVÁ, et al. (1999). "Effect of rosemary extracts on the stabilization of frying oil during deep fat frying." Journal of Food Lipids **6**(1): 13-23.

Rodríguez-Rojo, S., A. Visentin, et al. (2012). "Assisted extraction of rosemary antioxidants with green solvents." Journal of food engineering**109**(1): 98-103.

S

Salta, F., A. Mylona, et al. (2007). "Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract." Food Science and Technology International **13**(6): 413-421.

Sarkic, A. and I. Stappen (2018). "Essential oils and their single compounds in cosmetics—a critical review." Cosmetics **5**(1): 11.

Senanayake, S. N. (2018). "Rosemary extract as a natural source of bioactive compounds." Journal of Food Bioactives **2**: 51–57-51–57.

Shylaja, M. and K. Peter (2004). "The functional role of herbal spices." Handbook of herbs and spices **2**: 26-45.

Švarc-Gajić, J., Z. Stojanović, et al. (2013). "Development of a microwave-assisted extraction for the analysis of phenolic compounds from Rosmarinus officinalis." Journal of food engineering**119**(3): 525-532.

T

Tinsson, W. (2010). Plans d'expérience: constructions et analyses statistiques, Springer Science & Business Media.

V

Veillet, S., V. Tomao, et al. (2010). "Ultrasound assisted maceration: An original procedure for direct aromatisation of olive oil with basil." Food Chemistry **123**(3): 905-911.

W

Wang, Z., L. Ding, et al. (2006). "Improved solvent-free microwave extraction of essential oil from dried Cuminum cyminum L. and Zanthoxylum bungeanum Maxim." Journal of Chromatography A **1102**(1-2): 11-17.

Y

Yara-Varón, E., Y. Li, et al. (2017). "Vegetable oils as alternative solvents for green oleo-extraction, purification and formulation of food and natural products." Molecules **22**(9): 1474.

Z

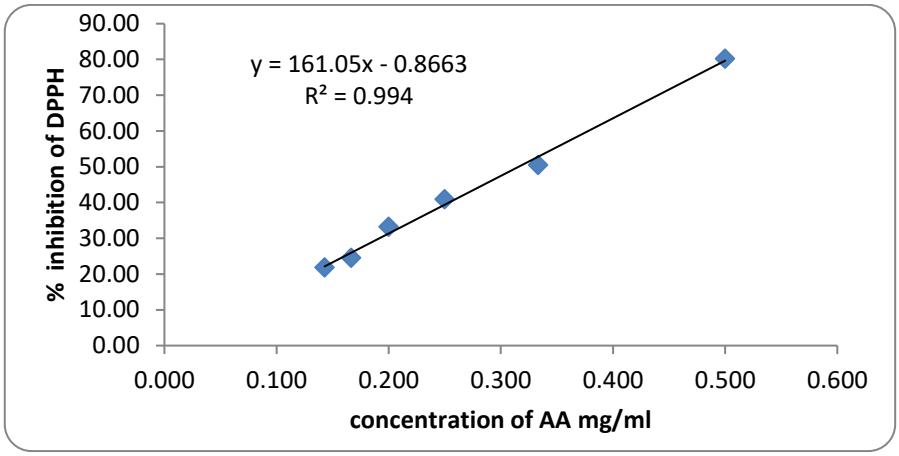
Zhang, H.-F., X.-H. Yang, et al. (2011). "Microwave assisted extraction of secondary metabolites from plants: Current status and future directions." Trends in Food Science & Technology**22**(12): 672-68

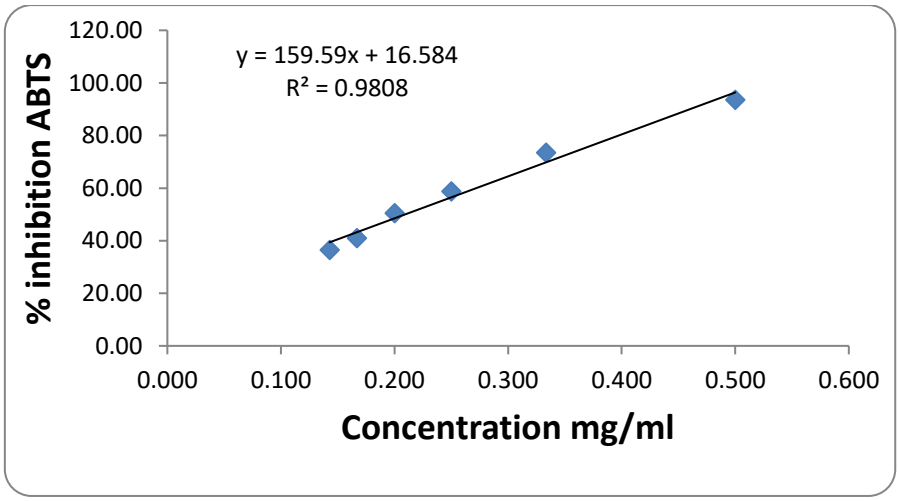
Zu, G., R. Zhang, et al. (2012). "Ultrasound-assisted extraction of carnosic acid and rosmarinic acid using ionic liquid solution from Rosmarinus officinalis." International journal of molecular sciences**13**(9): 11027-11043.

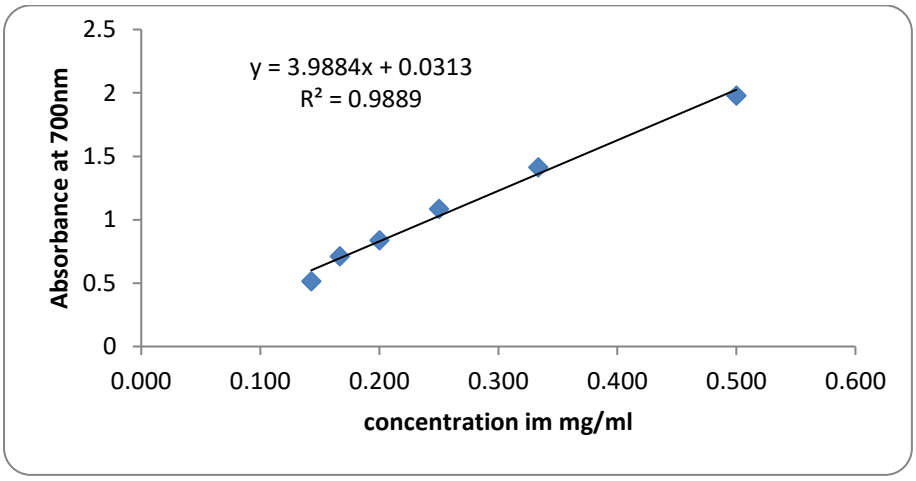
APPENDIX

Appandix 1

Ascorbic acid Etalon curves (DPPH, ABTS, and REDUCING POWER)

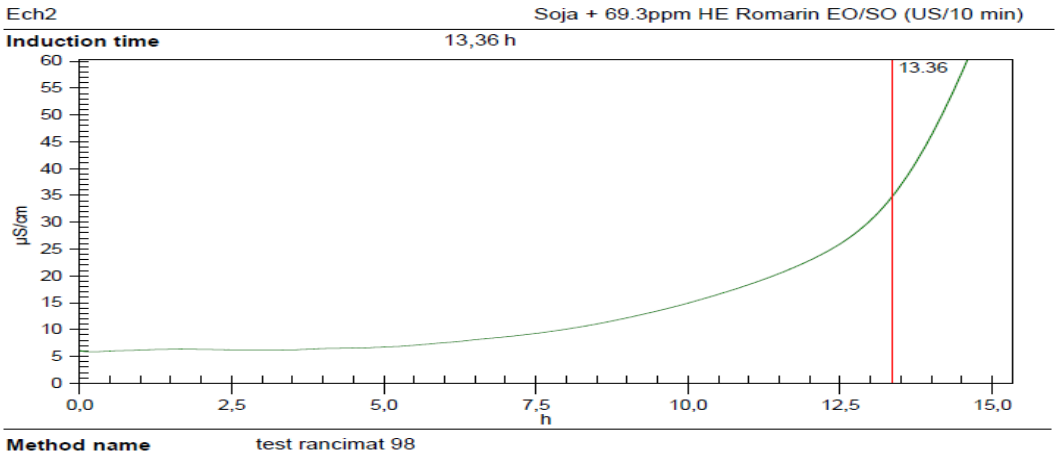


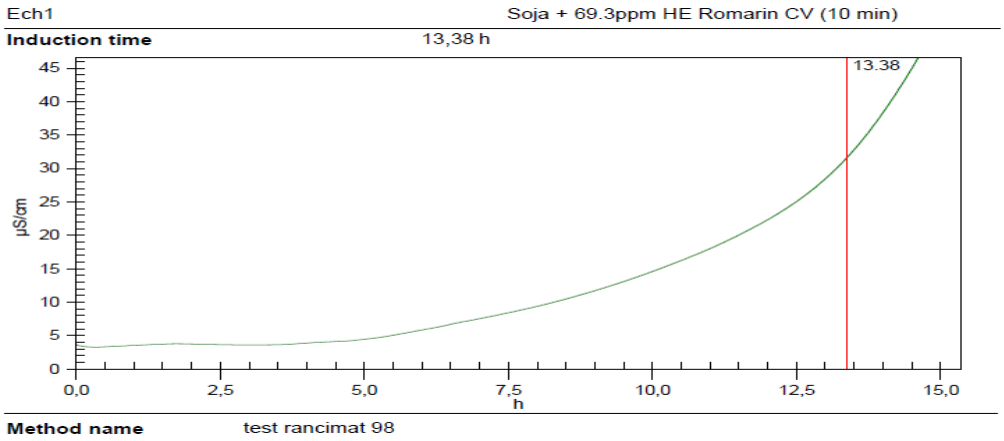


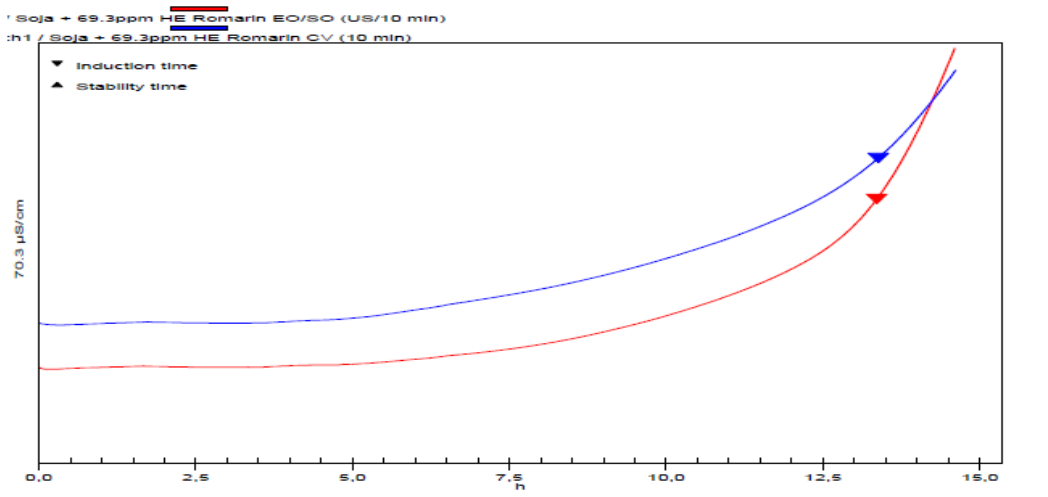


Appandix 2

Rancimat results for 69,35 ppm EO CM,UAE and comparison for







Abstract

In our study, we used four methods, HD, SD, MAHD and US-MAHD for the extraction of rosemary's essential oil from aerial part of the plant. The US-MAHD shows the best yield of extraction compared to the others methods.

The different oils extracted was submitted to the antioxidant activities, DPPH, ABTS and reducing power, the results obtained shows that the US-MAHD have a great power of antioxidant activities, so the latter was selected to be the best methods for extraction and enrichment of soybean oil.

The effect of two independent variable, Ratio and time, of UAE and CM was estimated by using response surface methodology. The optimum condition of enrichment to obtain a better antioxidant activity in UAE was 69,35ppm for ratio and 10min for time. An RBD (refined Bleached and Deodorized) soybean oil obtained from Cevital group (Bejaia) was enriched by the essential oil. The induction time using Rancimat method, Polar compound and radical scavenging activity was determined. Rancimat results was 13,88h for the SO-REO, for the polar compound the enriched oil was improved to 10h compared to 4h with unenriched one, the DPPH (%) was 73% for the SO-REO and 58% for the SO.

Keywords: DPPH, essential oil, rosemary, Rancimat, polar compounds, optimization, microwave assisted extraction, soybean oil.

Résumé

Dans notre étude, nous avons utilisé quatre méthodes, HD, SD, MAHD et US-MAHD pour extraire l'huile essentielle de romarin de la partie aérienne de la plante. US-MAHD présente le meilleur rendement d'extraction par rapport aux autres méthodes.

Les résultats de cette étude ont été rapportés comme étant de la plus haute importance dans le domaine des activités antioxydantes, DPPH, ABTS et du pouvoir réducteur, les résultats obtenus montrent que l'US-MAHD possède un grand pouvoir d'activités antioxydantes, c'est pourquoi ce dernier a été sélectionné aux meilleures méthodes d'extraction et d'enrichissement de l'huile de soja.

L'effet de deux variables indépendantes, Ratio et heure, des EAU et de la CM a été estimé à l'aide de la méthodologie de la surface de réponse. La condition optimale d'enrichissement pour obtenir une meilleure activité antioxydante aux EAU était de 69,35 ppm pour le rapport et de 10 minutes pour le temps. Une huile de soja RBD (blanchie et désodorisée raffinée) obtenue du groupe Cevital (Bejaia) était enrichie en huile essentielle. Le temps d'induction en utilisant la méthode Rancid, le composé polaire et l'activité de balayage des radicaux a été déterminé. Les résultats Rancimat étaient de 13,88h pour le SO-REO; pour le composé polaire, l'huile enrichie était améliorée à 10h comparée à 4h pour un composé non enrichi, le DPPH (%) était de 73% pour le SO-REO et de 58% pour le SO.

Keywords: DPPH, essential oil, rosemary, Rancimat, polar compounds, optimization, microwave assisted extraction, soybean oil.