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

MASTER

Thème

Enrichissement d'huile raffiné par la graine de *Myrtus Communis*

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List of abbreviations

ANOVA: Analysis Of Variance

Aw: Activity of water

DO: Optical density

DPPH: 2,2-Diphenyl-picrylhydrazyl

DW: dry weight

GAE: Gallic Acid Equivalent

M: Molar

MAE: Microwave Assisted Extraction

RSM: Response Surface Methodology

TPC: Total Phenolic Compounds

v/v: volume/ volume

BBD: Box Benken Design

G: gramme

µl: micro liter

ml : mililter

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Introduction

Introduction

The use of medicinal plants as therapeutic agents is as old as the presence of human life on earth. Although we are in the century of industrially synthesized medicines, medicinal plants are still used in various countries for their therapeutic effects .These plants represent an immense reservoir of potential compounds attributed to secondary metabolites and possess a very wide range of biological activities **(zeghad N, 2008).**

It y' has a certainty that during the use of a good part of a plant is regarded as waste, namely the air parts of the latter. however this waste would gain with being called by-product because it is a good source of the phenolic compounds of high-quality which can be used in traditional and modern medicine, thanks to their capacity to neutralize the free radicals in the biological systems, like in agribusiness industry with an aim of minimizing the use of synthetic antioxydant in food because of their undesirable effects on the human health.

Myrtus. communisis widely used in herbal medicine in Mediterranean countries, and recently determined to contain very high amounts of hydrolysable tannins and flavonoid glycosides (Tattini et al.,2006). These compounds possess biological functions in plants andexhibit preventive effects in animals and humans against majordisorders like cancer, cardiovascular diseases, arteriosclerosis,macular degeneration, and other age related disease. The direct enrichment of edible oil in carotenoids improve theedible oil quality with compounds beneficial to human health.The composition of the myrtle extracts depend, the part extracted and the extraction technique involved. There are three conventional ways for the enrichment ofedible oils through the addition of bioactive compoundsextracted from plants. The first involves a solvent extraction

of the plant material. The second method is throughsteam distillation of the essential oil contained in some aro-matic plants.The third technique is conventionalmaceration. All these method have the problems include thelong time and low yield of theextraction assisted by ultrasounds which is regarded as a process, showing an important positive effect on the extraction of the phenolic compounds with part of various vegetable sources, the phenomenon of cavitation being dominating. With a reduced time, a minimization of energy. UAE is considered more powerful than maceration **(Galvan and Al, 2013)**

We propose a work of enrichment here, by using the oil refined of soya with fruit product of myrtle, using ultrasound assisted extraction with use of the technique of experimental designs for a better optimization of the experimental conditions.So, the aim of this present work was to enrich low quality edible oil by using the antioxidant properties of bioactive substances of ***Myrtuscommunis*** seeds.

The second aspect is devoted to the optimization of the parameters of extraction (time, temperature, and diameter) with an experimental design using extraction assisted by bath ultrasounds.

The third aspect is of a nature phytochemical based mainly on the extraction and the quantification of the TPC, chlorophyll and carotenoids by colorimetric method, it also relates to an evaluation of the antioxidant activity , index of peroxide and acidity.

It is a study realized at university A/MIRA of BEJAIA at laboratory BBBS.in order to better locate the context in which enrolled this modest work.

Theoretical

Part

Chapter I

Myrtus communis

I. MYRTUS COMMUNIS

I.1 Origin and history of myrtle

Myrtus communis L. is a traditional plant from the Corsican coast. It is a shrub, one to three meters tall, with bright green leaves and white flowers during the blossoming season (June to July). The fruit is spherical in shape, dark blue to black in colour, edible but astringent, and grows in autumn **(Gamisans and Jeanmonod 2007)**. Myrtle is included in the *Myrtus* species of *Myrtaceae* family which is a large family containing approximately 132 genera and 5671 species **(Govaerts et al 2008)**. Like all evergreen plants with aromatic, it was also a counting a symbol of strong life and force (**Heilmeyer ,M.,2007)**.

In additional, this species is regard significant aromatic plant because of high content of essential oil in different parts, such as leaf, flower, and fruit glands, due to the existence of rules and genetic fixed materials, the chemical composition is differed according to the seasons **(Fadi et al 2012)**.

Figure 1: photography of myrtle berries (*myrtus communis*)

I.2 Geographical and distribution

Distribution Myrtle (*M. communis* L.) is a common part of typical Mediterranean flora. The plant grows abundantly from the north western to the eastern Mediterranean, including bordering countries and western Asia, as well as Aegean regions **(Baytop 1997).** Myrtle's native to southern Europe, North Africa and west Asia. It is also distributed in Southern America, northwestern Himalaya and. Australia. Myrtle is cultivated in gardens, especially in North west Indian region, because of its fragrant flowers **(Nadkarni 1989)**. which possesses the same vegetative characters. the morphological difference between the two varieties regards to size of fruits and leaves. This herb grows spontaneously Iran,

Spain, France, Greece, Turkey, Algeria, Morocco, Croatia and Montenegro **(Naserian 1997)**

I.3 Etymologies and classification of the myrtle plant

Myrtle is included in the *Myrtus species* of Myrtaceae family which is a large family containing approximately 132 genera and 5671 species **(Govaerts et al 2008).**

Kingdoom	plantae
division	spermaphytae
Class	dicotylédona
Order	myrtales
Family	myrtaceaes
Genus	myrtus
Species	myrtus communis L

I.4 Chemical composition of *Myrtus communis* L. extracts

Main secondary metabolites are polyphenol sand essential oils. Myrtus species have been reported as very rich involatile oils **(Satrani et al. 2006),** phenolic acids and flavonoids **(Romani et al. 1999)**, tannins **(Diaz and Abeger 1986),** anthocyanin pigments **(Martin et al. 1990)** and fatty acids **(Cakir2004)** as show in table (1).

Table 1: Chemical composition of *Myrtus communis L* in deferent part of myrtle

***M. communis* L part**	**Composition**	**references**
Leaves 	Contain 1,8-cineole(13.5–19.6%), linalool (7.7–15.8%), linalyl acetate (2.5–6%), terpineole, terpinolene, tannins and flavonoid compounds	**(Chryssavgi et al. 2008).**
Berries 	Berries are composed of tannins anthocyanins (0.2–54%), fatty and organic acids (9–52%), and its content depends on used extraction solvent and/or ripening period It is evident that the content of these compounds also differs	**(Messaoud et al. 2012).**
leafs and Flowers  	Leafs and flowers contain essential oils, phenolic acids, flavonoids and tannins	**(Aidi Wannes et al. 2010).**
Seeds 	Seed extract had essential oils, hydrolysable tannins ((80.20%,) anthocyanins (69.36%, In all tests, the seed was the part with highest antioxidant potential.	**Aidi Wannes,W.,2016**

I.5 Utilization of myrtle

Myrtle's leaves and fruit are traditionally used as antiseptic, disinfectant, and hypoglycemic agents (**E l f e l l a h et al. 1984).** It is one of the most widely used and well- documented medicinal plants in the world (**Clark 1996; Gortzi et al. 2008; Messaoud et al. 2012).** antiseptic and anti-inflammatory activities (**Diaz, A. M.et al 1987).** Different parts of the plant find various uses in food and cosmetic industries (**Chalchat J. C. 1998)**

The essential oils that called volatile oil mainly consists of Limonene, α-pinene, β-pinene, and linalool. These oils act as anti-bacterial properties, antifungal and antiviral, reducing the level of cholesterol, as well as antioxidant properties in cells it has been found **. (Blumenthal, 2002)**

Myrtus communis, belonging to the Myrtaceae family, is a pleasant annual shrub with dark blue ripe berries, which have a long history of application in the perfumery, cosmetic, food and pharmaceutical industries **(Nuvole and Spanu 1996)** Food preparation – liquors, flavoring meat and sauces; Medicine – used also orally for infectious disease such as diarrhea and dysentery and externally for skin diseases and wound healing healing(**Messaoud et al. 2012),** Medicine – agains varicose veins and for preparing capillary lotions for external use **Le Floch (1983).** Medicine – remedy for asthma, eczema, psoriasis, diarrhea, gastrointestinal disorders and urinary infections, administrated orally; applied by inhalation and externally (**Ziyyat et al. 1997)**

I.6 Phenolic compounds: chemical structure and properties

I.6.1 Defition of phenolic compounds

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins **(Han et al., 2007).**

Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet **(Dai and Mumper, 2010; Liazid et al., 2007).**

They drive biogenetically from two main synthetic pathways: the shikimate pathway and acetate pathway. Chemically, polyphenols are a large heterogeneous group of compounds characterized by hydroxylated phenyl moieties posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer **(Balasundram *et al*,. 2006).**

The main classes of these compounds comprise flavonoids, phenolic acids, and tannins, among others. Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds **(Martins *et al*,. 2011b).**

I.6.2 Roles of phenolic compounds

Phenolic compounds play an important role in plants, foods and humans.

I.6.2.1 In plants

Phenolic compounds play an important role in plants, these compounds carry out diverse functions, such as protective agents against UV light, take part in growth and reproduction, components of pigments, essences, flavours and they contributing to color, astringency and bitterness of fruits and vegetables, and frequently serve as pigments in plants to attract pollinators and as plants' chemical defence mechanism against infections caused by microorganisms and injuries by insects **(Soto *et al*,. 2011).**

I.6.2.2 In nutrition and human physiology

Although the mammalian body has certain defense mechanisms to combat and reduce oxidative damage, epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant phytonutrients notably flavonoids and other polyphenolics is advantageous for the health. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. In recent studies, a growing interest in biology and medicine has been focused on oxidative stress. Research has pointed out that the most effective method to reduce oxidative stress is antioxidant supplementation **(Esfahlan *et al*,. 2010**).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidising chain reactions .There is a great interest in the food industry because they improve the quality and the nutritional value of foods. When added to foods, antioxidants minimize rancidity, retard

the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life **(Antolovich *et al*,. 2002).**

I.7 Phenolic compounds from agro-industrial by-products

Special attention is focused on the extraction from inexpensive or residual sources from agricultural industries. By-products, remaining after processing fruits and vegetables in the food processing industry, still contain a huge amount of phenolic compounds. Some studies have already been done on by-products, which could be potential sources of antioxidants**(Balasundram *et al*,. 2006)**. Phenolic compounds with antioxidant activity have been identified in several agricultural by-products, such as rice hulls , buckwheat hulls and almond hulls **(Wojdyło *et al*,. 2007)**

I.8 Phenolic compounds of myrtles by- products (seeds myrtle)

Extracts of whole myrtle seeds possess potent free radical-scavenging capacities. These activities may be related to the presence of flavonoids and other phenolic compounds in seeds. The present work aimed to study the phenolics and antioxidant activity of the methanol extracts of *Myrtus communis* seed extract had higher total phenol content (23.87 mg GAE/g DW). Significant differences were also found in total tannin contents among different myrtle parts, representing 18.01 mg GAE/g DW in seed, the condensed tannin content was relatively low in all samples tested, the highest value being found in whole fruit extract (0.96 mg CE/g DW). The high-performance liquid chromatography analysis indicated that the main phenolic class was hydrolysable tannins (gallotannins) in seed (80.20%, 8.99 mg/g MS) while the pericarp was characterized by a predominance of anthocyanins (75.40%, 3.74 mg/g DW). In all tests, the seed was the part with highest antioxidant potential. **(Wannes, W.A. and Brahim marzouk 2012)**

Chapter II
Enrichment of Refining oil

II. Refining soya oil:

In a rough state, oils contain a certain number of impurities which can make them inedible. Some of these impurities have a harmful influence on the taste, the odor, the aspect of the product, its conservation and its toxicity. The purpose of the refining is to eliminate the undesirable substances in order to preserve the nutritional quality of the product and to avoid the formation of new chemical species. **(ANONYME1, 2000)**

Various types of refining can be used:

- Refining chemical
- Refining physics
- Refining enzymatic

II.1 Principles of the three types of refining

II.1.1 chemical refining

It includes the whole of the stages of demucilagination, neutralization, discoloration, and deodorization.

II.1.2 Physical refining

It consists in neutralizing the fatty corps by subjecting it to a drive with the vacuum vapor; it is what is called commonly neutralizing distillation **(Denise, 1992)**. This refining will be considered only if, exeptionnely, crude oil presents a low content of phospholipids in order to remove the production of pastes of neutralization (**Ericksson and Al, 1989)**

II.1.3 enzymatic refining

The enzyme used is a phospholipase A1 (Lecithase Ultra) is able to convert the hydratable and non-hydratable phospholipids into lysophospholipides which will be eliminated with the mucilages **(Novozymes, 2002).**

II.2 Components to be eliminated

Oils such as they are obtained after extraction, are not mixtures of pure triglycerides, they contain according to the source and the mode of extraction of the variable proportions of undesirable and harmful compounds**.** Thus crude oils must undergo a series of treatment of purification or refining in order to remove impurties **(Gibon and tirtiaux, 1998).**

The following table illustrates the various compounds eliminated during the refining.

Table: compounds eliminated during the refining

Nature of component	Origin	Disadvantages of their presence
Free faty-acide	Naturel components by hydrolysis	Taste, hydrolysis , organolyptic instability
phospholipides	Natural components	Turbid aspect,organoliptic instability, hot deposit and tanning
Products of oxidation	Self-oxidation	organolyptic instability color nutrition
flaveurs	Natural self-oxidation	Odor,taste
waxes	Naturel components	Turbid aspect
pigments	Naturel components	Color, organolyptic instability
Metals(iron,copper)	Natural components contamination	Catalysts of xidation
Contaminants Heavy metals Pesticides mycotoxins	contamination	Food hygiene health

II.3 Disadvantages of the refining:

In spite of its big role; the refining presents however certain disadvantages:

- It preliminary demucilagination can be imperfect under the action of the soda which completes the operation quickly.
- Oil Loss per drive in the pastes of neutralization, water of washing and the bleaching grounds **(Francois, 1974).**
- It quantitative modification of the composition of the oil to the damage of the essential nutrients.
- It destruction of the vitamin E which is essential with the prevention of oxidation.

- Inversion of the space configuration of the molecule of the fatty-acid around the double connection **(Debruyne, 1999).**

In optics to compensate for its losses and with a view to improve quality of the oil , an enrichment of the edible oils containing vegetable matrices.

Indeed, oils: sunflower, olive, soya, palm, colzas and groundnut were supplemented by the origan, the sauge, rosemary, spinaches… etc

II.4 Exogenous enrichment of extra virgin oil

There is a great interest in natural antioxidants for oil supplementation aimed at inhibiting oxidation and keeping nutritional and sensorial quality and/or to obtain oil with added-value. With regard to the enrichment step, there are three alternatives in the literature for oil enrichment with valuable compounds from plants:

- liquid-liquid extraction, in which the oil is put into contact with an alcoholic extract of phenols, which are transferred to the oily phase as a function of their distribution factor, removing the alcoholic phase by centrifugation;
- solid liquid extraction, in which the purified phenolic extract is dried under appropriate conditions and the paste obtained is partially dissolved into the oil as a function of
  the solubility of the different paste components in the oily phase
- combination of these procedures, in which the alcoholic extract and the oil are put into contact and the two phase system is subject to alcohol removal in a rotary evaporator **(Achat et al., 2012;)**. In addition, theses enrichment methods may be assisted by new technology as ultrasound to facilitate the enrichment procedure and better dissolution of the extract plant**.**

A dramatically reduced actually, a greener procedure was developed using ultrasound technology. In this procedure, as shown in the figure 7, plants were directly put into the oil and ultrasounds were applied to the mixture in order to enhance the speed of the maceration. This resulted in a greener procedure using oil as solvent (no petroleum solvent), run out at room temperature (to prevent thermal degradation) and in time **(Veillet et al., 2010).**

Experimental Part

ChapterIII
Material
and methods

There is a great interest in natural antioxidants for oil supplementation aimed at inhibitingoxidation and keeping nutritional and sensorial quality and obtain oil with added-value.With regard to the enrichment step, there are three alternatives in the literature for oilenrichment with valuable compounds from plants:

But what are the conditions that should be provided the transfer of these polyphenols?

The answer to this question will be the subject of the development of this experimental study.

The extraction, proportioning, as well as the quantification of polyphenols are carried out and the antioxidant capacity is measured .This present work carries known the enrichment of oil refined by the phenolic compounds of seeds of *Mytus Communis.*

Then an optimization of the parameters of enrichment is carried out to choose the outline levels of experiments to adopt, followed launching of this last. Thus the enrichments dictated by this plan are accomplished. The extraction, proportioning, as well as the quantification of polyphenols are carried out and the antioxidant capacity is measure

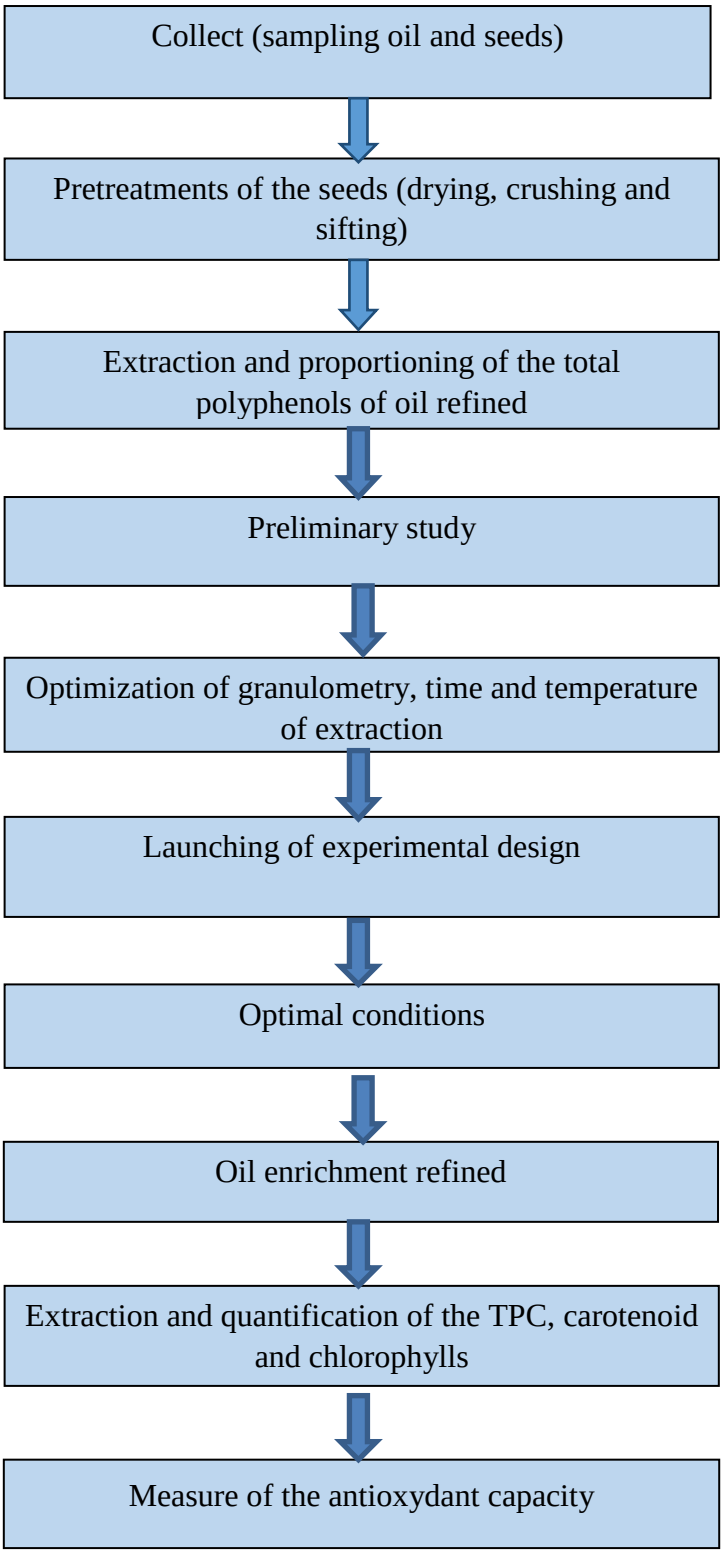


Figure2: general outline of the scheme of work

III.1. Plant material and preparation samples

Myrtus communis, were collected in the area of Adekar (Bejaia. Algeria) in December 2016. After the separation of the by-products (seeds) from myrtle were collected at optimal maturity (January), from Adekar (Bejaia, North-east of Algeria). Fruits were isolated manually from the aerial parts. It has been washed with a tap and distilled water to remove any adhering soil and dust. Fruits sample were dried with microwave drying – ultrasound assisted pretreatment method (4 ml of water was needed for 1g of seed sample and placed in an ultrasonic bath during 90mn then in microwave at 500W). Then the seed were separate to pericarp and ground with an electrical grinder (IKA model A11 Basic, staufen, Germany), and sieved through standard 250µm size. Two fraction of seed sample with particle size < 250, 125µm were stored in airtight bags until use.

Table3: characteristic of samples analysis

Staded Part	Origin	collected date	Characteristic
Seeds	Adekar	17/12/2016	The myrtle fruits (0,8-1.2cm) are multi-seeded berries (4-12seeds)r with different shapes (ovate, oblang, pyriform,or round)

III.1.1.Determination of water content

Principle

The water content was determined by the method described by **(Schössler, K., H. Jäger, et al., 2012).**A test specimen of 2 G of the powder of the fruit is put in a drying oven at 103°C ± 2°C until obtaining a constant weight.

Expression of the results

The water content (H %) is calculated by the following formula Where:

H%: Water content in %

M1: Mass capsule + fresh matter before drying in g

M2: Mass of the unit after drying g

P: Mass test specimen in g.

$$H\% = \frac{M_1 - M_2}{P} \times 100$$

III.1.2. Activity of water

The water activity of sample was determined by the WA meter apparatus presentedin the figure. A. Weight of 2 to 4g of sample was placed in a small dish into the apparatus,and then the lid of the room of samples was closed. After stabilization of the measures the water activity of the sample was read.

Water activity of the powder (125µm) of the myrtle seeds was 0.235 at 26°C.and powder (250µm) was 0,302 at25C°.

Figure 3: WA measures

III.2.Enrichment vegetal oil procedure:

The enrichment procedure involved immersing dry plants in the vegetal oil under appropriate conditions in order to transfer some properties or compounds from the plants (myrtle seeds) to the oil, obtained by average mechanics and chemical starting from soya.

III.2.1. Instrumentation

An ultrasonic bat (Ctra.NII:585 Abrera (Barcelona) Spain, Ultrasound H-D, frequencies: 20 to 60 KHZ, Power: 80 to 600 W) was utilized for enrichment process (Figure 4).

Figure 4 : Enrichment oil in bath ultrasound

III.2.2. Extraction of the polyphenols from the enriched oil:

Extraction of the phenolic compounds is carried out before and after enrichment.the adopted protocol that is described by (**Kalantzakis et al., 2006).**

Principle:

It is a liquid / liquid extraction which consists of dissolving the oil in an organic solvent and mixed with a polar solvent for the recovery of the phenolic compounds. **Appendix (2,3).**

Procedure:

A test portion of 2.5 g of oil is added to 5 ml of hexane (99%) and 5 ml of methanol / water (6/4, v / v), then vortexed for 2 min, the mixture is centrifuged at 3500 rpm for 10 min. The polar fraction (methanol phase) is recovered, while the apolar (hexanic) phase is depleted. The two fractions obtained are mixed and stored at 4 ° C. and protected from light **(Kalantzakis et al., 2006).**

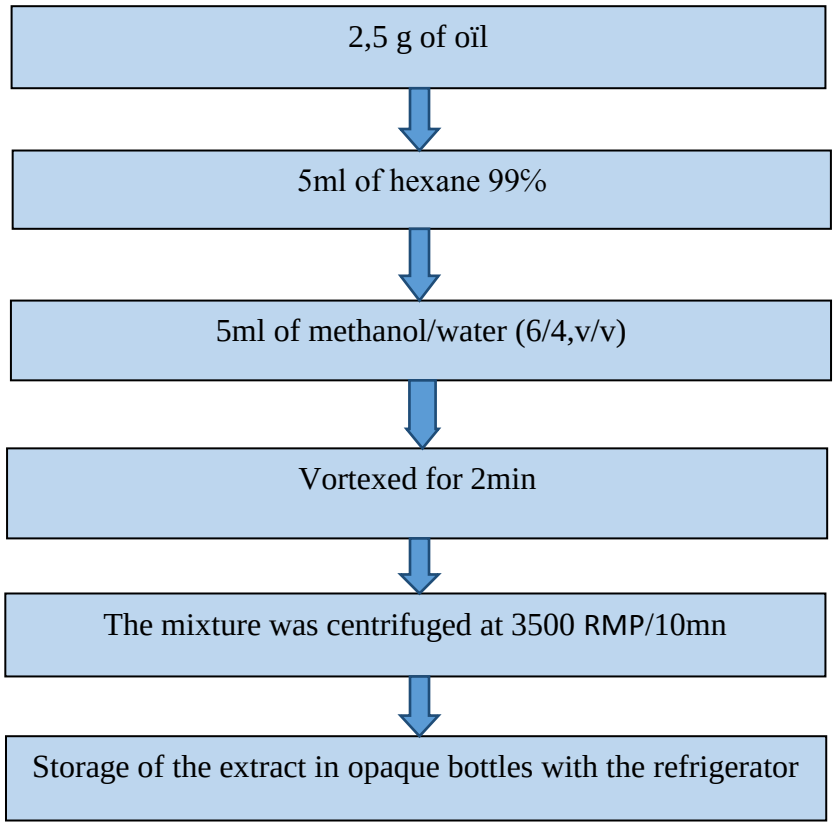


Figure 5: Protocol of extraction of the polyphénols starting from oil

III.3.Phytochemical analysis

III.3.1Determination of Total phenolic content

Total TPC concentration was estimated by Folin-Ciocalteu's assay, with absorbance monitored at 760 nm **(Georgé et al., 2005).** The spectrophotometric measurement was repeated three times for each extract and the average data was interpolated in a gallic acid calibration curve andexpressed as mg of gallic acid equivalents per g of dry weight sample (mg GAE g-1DW)

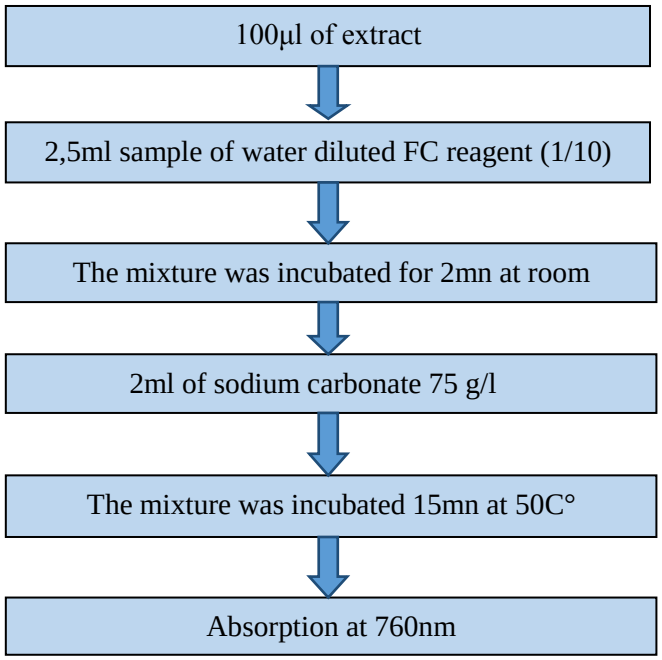


Figure6: diagram representative of the protocol of proportioning (**Georgé et al., 2005).**

III.3.2. Determination of carotenoids and chlorophyll content:

Chlorophyll and carotenoid pigments were determined by UV spectroscopyat 670 and 470 nm, respectively, in cyclohexane, using thespecific extinction coefficients, according to the method proposed by According to **Minguez Mosquera et al (1991),** the protocol for the determination of carotenoids and chlorophyll is as follows:

- A sample of 7.5 g of oil is adjusted to 25 ml of cyclohexane in a graduated flask. The maximum absorbance at 670 nm and at 470 nm gives information on the chlorophyll fraction and the carotenoids respectively. The value of the specific extinction coefficient used:
- E0 = 613 for Pheophytine (major component of chlorophylls).
- E0 = 2000 for Lutein (major component of carotenoids).

Results:

$$Chlorophylle\ (mg/Kg) = \frac{A_{670}-10^{-6}}{613-100\times T}$$

$$Caroténoïdes\ (mg/Kg) = \frac{A_{470}-10^{-6}}{2000-100\times T}$$

A: absorbance.

T: optical path (thickness of the vessel 1 cm)

III.4. Optimization of the extraction parameters by the "Box-Behnken Design" method:

In a context of valorisation of plant products, an experimental methodology for the extraction of bioactive substances from the after mentioned plant material has been put in place. The first part of this study is devoted to the investigation of various factors influencing the extraction process and to determine the levels (low and high) of each factor retained or controlled by the experimental design (JMP®). The second approach consists in choosing the appropriate experimental plan for the study. In other words, the experimental method chosen should facilitate the interpretation of the results. It must also minimize the number of attempts without altering the quality.

The Response Surface Methodology (RMS) is an effective tool to optimize a process. The strength and advantage of this technique is the reduced number of experiments required to evaluate multiple parameters and their interactions. Consequently, it consumes less time and work than other approaches required to optimize a process (**Xinpeng et al., 2006).**

III.5. Preliminary study:

Prior to optimization, experiments were carried out to determine the appropriate range of conditions for the extraction of phenolic compounds from the seeds myrtle ,diameter ,temperature ,and times of extraction time, mass / volume ratio And the extraction power by changing one independent variable at a time while maintaining the other constants (**Chan etal., 2009).**

III.5.1. Choice of the temperature of extraction

A test specimen is carried out to determine the best temperature of extraction, according to traditional model of extraction by varying the parameter (temperature) and fixing the others (diameter and time)

Table 4: optimization of temperature extraction

Temperature(°C)	**15**	**25**	**35**	**45**
Time(min)	Time was optimized 30mn			
Diameter(µm)	**250 µm**			

III.5.2.Choice of the time of extraction

A test specimen is carried out to determine the best time of extraction, according totraditional model of extraction by varying the parameter (time) and fixing the others (diameter and temperature)

Table 5: optimization of times extraction

Time(min)	15	30	45	60
temperature(c°)	Best temperature of extraction 35°			
Diameter(µm)	250			

III.5.3.Choice of granulometry

A sample is carried out to optimize granulometry, according to the model traditional of extraction after optimization of temperature and time of extraction, while varyinggranulometry and fixing the parameters (time, and temperature) table, the granulometry of the powders of myrtle seeds was determined by sifting. The selected sieves have the following diameters: 250 and 125 µm. The powders thus obtained are preserved in sealed containers and are stored in the dark.

Table 6: Optimization diameter extraction

Diameter(µm)	125	250
Time(min)	Best time of extraxtion30min	
Temperature(c°)	Best temperature of extraction	

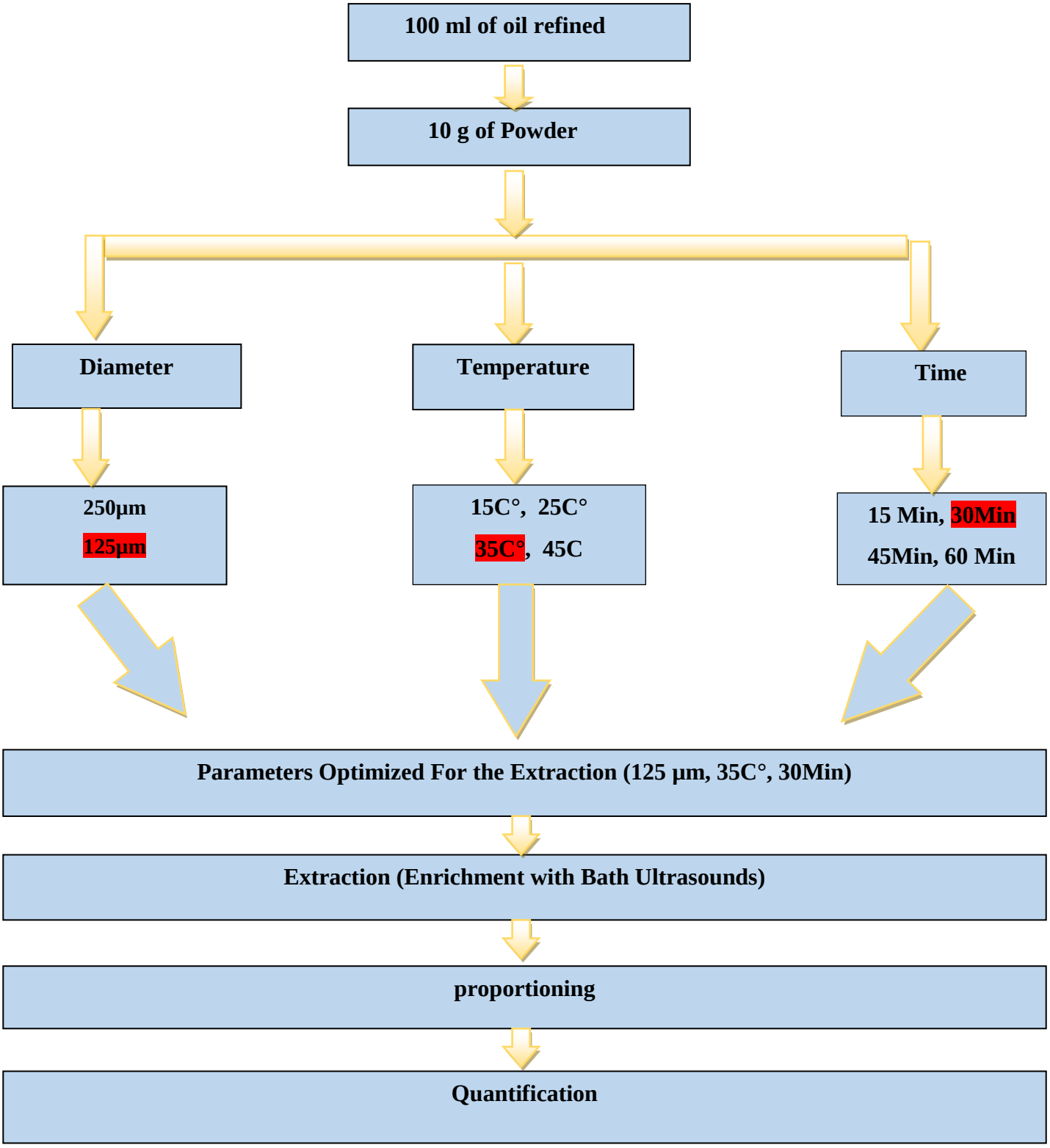


Figure7: Summary diagram of the preliminary study

III.6. Experimental Design (Box-Behnken Design):

Box-Behnken Design (BBD) is a second-degree multivariate model, it is easy to implement and possess sequentiality property. Its main characteristics are (**bezerra et al., 2008):**

- 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.

In our study, we applied a three-level Box-Behnken plan to evaluate the combined effect of four independent variables: time, temperature, diamitre, which are designated X1, X2 and X3 respectively. The preliminary study we carried out allowed us to determine the low and high levels for the variables influencing the experimentation which are illustrated in the table opposite. Le plan de Box Behnken utilisé est représenté dans le tableau x.

Table 7: Experimentation matrix

Experience		Temperature of extraction (c°)	Temps of extraction (mn)
+-	1	45	15
00	2	30	32,5
0A	3	30	50
00	4	30	32,5
00	5	30	32,5
0a	6	30	15
00	7	30	32,5
-+	8	15	50
a0	9	15	32,5
++	10	45	50
A0	11	45	32,5
--	12	15	15

According to formula (1), 12 experiments will be carried out in order to estimate the mathematical model of the response investigated.

The various responses, data analyzes and interpretations will be carried out using the experimental design. The methodology of the surfaces of answers will make it possible

to model the answers studied in the form of a polynomial equation of the second degree below:

$$y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{34}X_3X_4 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{24}X_2X_4 + a_1X_1^2 + a_2X_2^2 + a_3X_3^2 + a_4X_4^2 + E$$

Avec :

a_0, a_1, a_2, a_3, a_4, a_{12}, a_{23}, a_{34}, a_{13}, a_{14} et a_{24} : sont les coefficients de régression, X_1, X_2, X_3 et X_4 : sont les facteurs étudiés ;

y : la réponse mesurés ;

E : l'erreur.

III .7.Antioxidant activity

III .7.1.DPPH essay:

The antioxidant activity of the extracts was estimated by the DPPH method,according to the procedure described by (**Dudonneet al. (2009).**

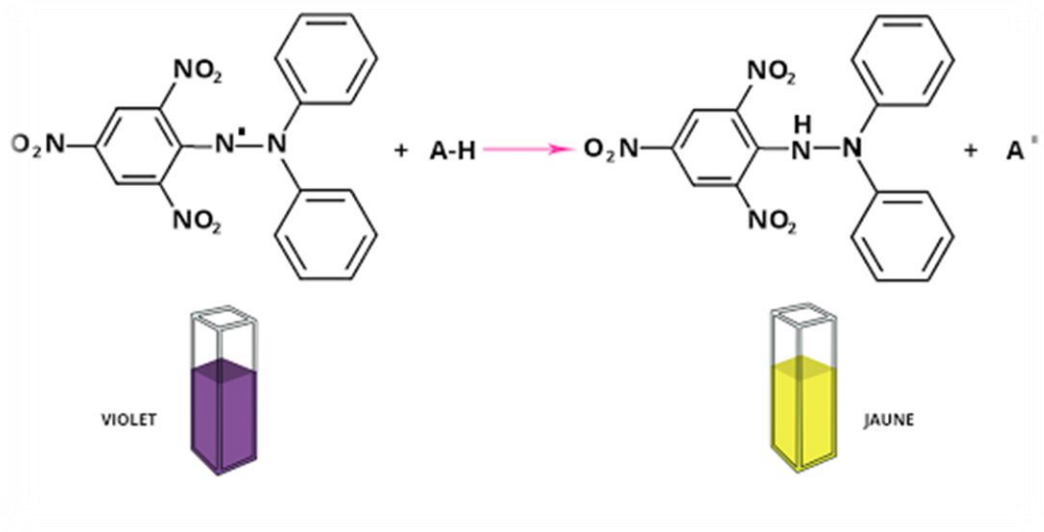


Figure8: Reaction of polyphenolicstructure with the radical DPP

Principle

The chemical compound 2,2-diphenyl-1-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl) was one of the first free radicals used to study the antioxidant structure-activity relationship of phenolic compounds. Since this compound possesses an unpaired electron on a nitrogen bridge atom, the molecules of the radical do not form dimers, so it remains in its relatively stable monomer form at room temperature. It is this delocalization which causes the coloring Dark violet characteristic of the DPPH solution. Measuring the

decrease in DPPH staining makes it possible to measure the effectiveness of an antioxidant, due to a recombination of the DPPH radicals which has a maximum absorbance at 515 nm. For phenolic compounds, the main mechanism of action is the scavenging of free radicals by transfer of the H atom onto the DPPH ° then transformed into a stable DPPH molecule.

The results may be expressed as anti-free radical activity or as a percentage of free radical inhibition using the following formula:

$$\%\ Scavenging = \frac{\left(A_{control} - A_{extract}\right)}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of DPPH• att = 0min;$A_{extract}$ is the absorbance of DPPH•in the presence of the

Procedure

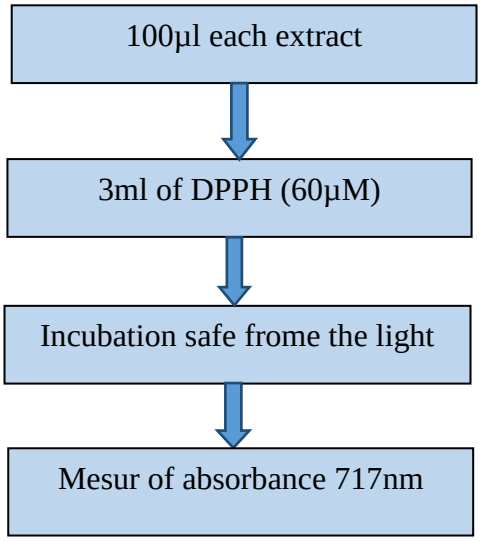


Figure 9: Analytical protocol used for determination of antioxidant activity

III.7.2. reducing power

Principle

The yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+}can be monitored by the measurement of the absorbance at 700 nm **(Zou et al., 2004)**.

One mL of different extracts was mixed with 2.5 mL of a 0.2 M (m/v) sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (m/v) Potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated in a water bath at 50° C for 20 min. Then, 2.5 mL of 10% (m/v) trichloroacetic acid were added. Finally, 1mL of the obtained solution was added to 5 mL of distilled water and 1mL of 0.1% (m/v) ferric chloride ($FeCl_3$), the intensity of the blue green color was measured at 700 nm. Tests were carried out in triplicate.

Chapter IV
Results and discussions

IV. Results and discussion

IV.1.Water content and activity of water (Aw):

IV.1.1.The water content:

The water content of the fresh sample *myrtus communis* is of 43, 65% what explains why almost of vegetable material weight fresh east constitutes water

Moisture supports well the development of the micro-organism and the mould which involve fast degradation of vegetable material at the court of conservation

IV.1.2.The activity of water Aw

The water content and the activity of water (Aw) play a big role in the conservability of the powder of the dried fruits. Moreover, the vegetable matrix to incorporate in a oil matrix, should preferably contain a small quantity of water to avoid possible hydrolysis of triglycerides, which could consequently release from the more oxydable free fatty-acids.

The activity of the water of the powder of seed of *Myrtus Communis* is of 23% this low water content makes it possible to better preserve the oxidizing anti properties of the total compounds phenolic present in the powder.

IV.2. Preliminary study

The results obtained by the preliminary study as shown reveal that the quantity of TPC compounds extracted is essentially dependent of the temperature and time factors.
The highest concentration of TPC (73, 92 ± 7, 25 mg EAG/kg oil), was obtained for 35°C.
Before having recourse to a plan of experience, different parameters are to be taken into account in our analysis.

IV.2.1.Effects of the temperature

- **Effect on the TPC**

As fixed time and diameter factors the temperature was varied (15, 25, 35, 45 °C) the results obtained of the TPC contents was illustrate in the following figures.

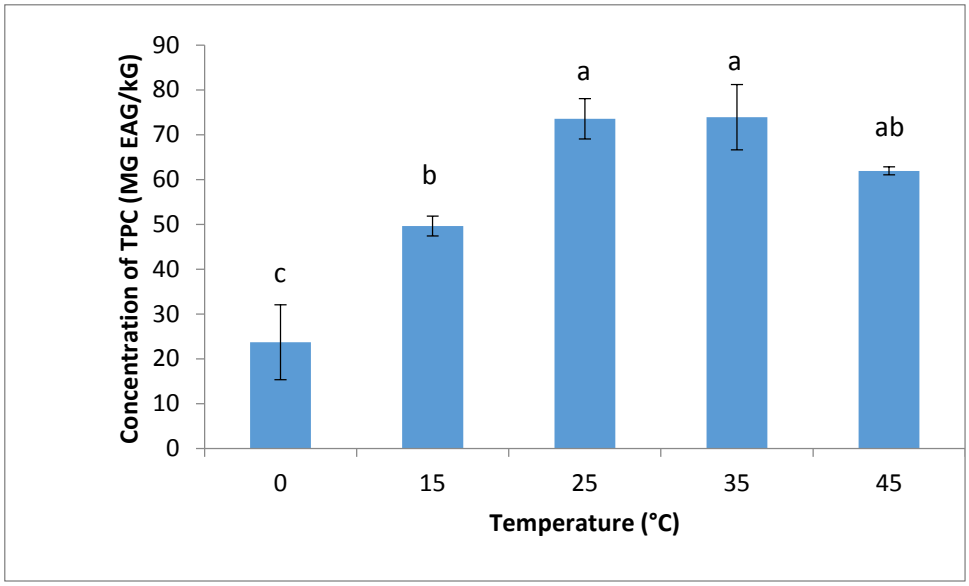


Figure 10: Effect of the temperature on TPC yield from enriched oil using Ultrasound assisted extraction.

Values marked by different letters are significantly different (p<0.05).

The yield of TPC increased significantly with increasing of the temperature in the extraction medium; up to35c° with a higher yield at 73,926mg/kg. However, TPC yield began to decline with an increase of temperature 45°C(61,925mg /Kg) that can be due to the degradation of the TPC higher temperature (**larrauri et al 1997**).

- **Effect on the carotenoids**

According to the results obtained in the previous figure, the carotenoids increase with increasing temperature at (45°C) , that obtained the higher yield (45 mg/kg). The result is explained by the fact why the carotenoids are not affected by the temperature up to (45°C).

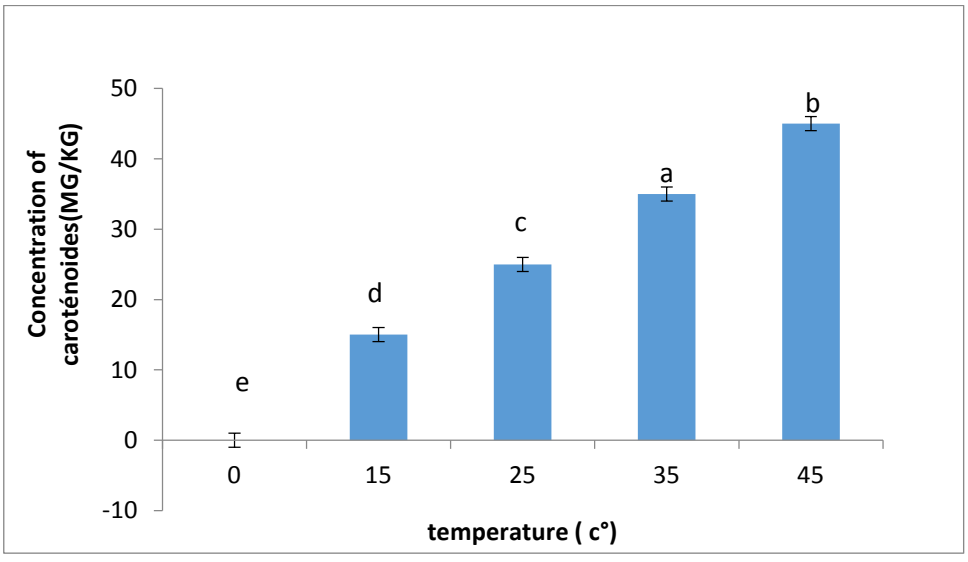


Figure 11: Effect of the temperature on the carotenoid content

Values marked by different letters are significantly different (p<0.05).

- **Effect on the chlorophylls**

According to the results obtained in the previous **(figure 12)** ,the chlorophylls contents increases with increasing temperature at (35c°) that obtained the higher yield (3.85 mg/kg) However, chlorophylls yield began to decline with an increrease of temperature(45c°) , that which can explain by the degradation of chlorophyll at higher temperature. The temperature was examined at levels between 15 and 45°c for the optimization design. The temperature at 35°C was chosen for the determination of optimal extraction time diameter of powder.

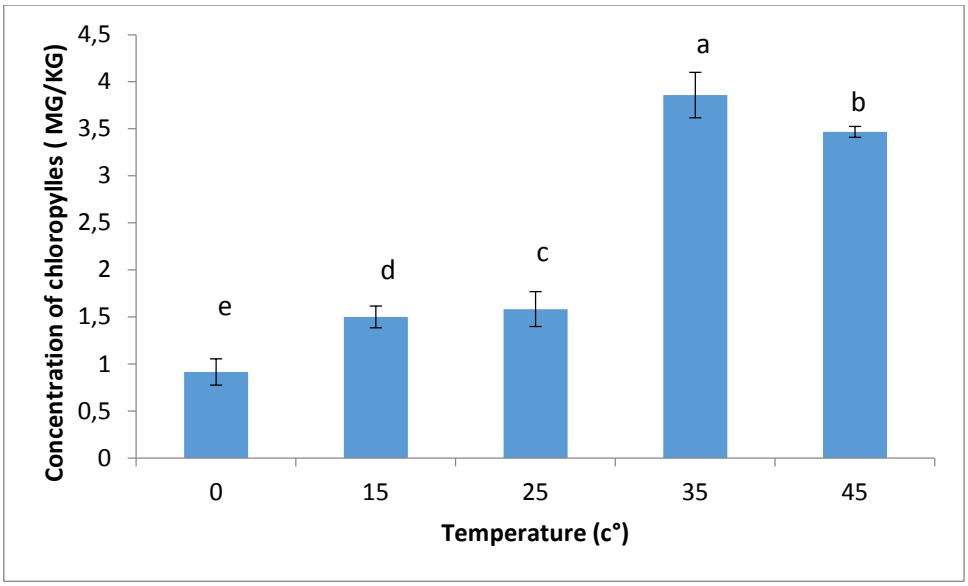


Figure 12: Effect of the temperature on the chlorophyll content for enriched oil using ultrasound assisted extraction

Values marked by different letters are significantly different (p<0.05).

IV.2.2. Effects off the time

The time of extraction is another important parameter which influences the extraction of the phenolic compounds and the pigments (**Tan et al.,2013, Farid et al., 2014)**.

The effects of sonification time on the recovery of TPC carotenoid and chlorophyll from Enriched oil was investigated at levels ranging from 15 to 60min with fixed temperature at 35°C and powder diameter at 250µm was showed in the following figure respectively.

- **Effect on the TPC:**

After (minute 15) of TPC contents increased significantly at (34.67 to (73,34mg/kg) with 30min,.than a higher decrease was observed at (60min) with TPC content at (43.mg/kg).

there is the risk of the degradation of extracted compounds at higher time extraction .Our results are in agreement with the findings **(Haque 1999**). Similar observations were also reported by **(Hossainn, O.N.G and al.2012**) during the optimization of water of composed of antioxydant of marjoram.

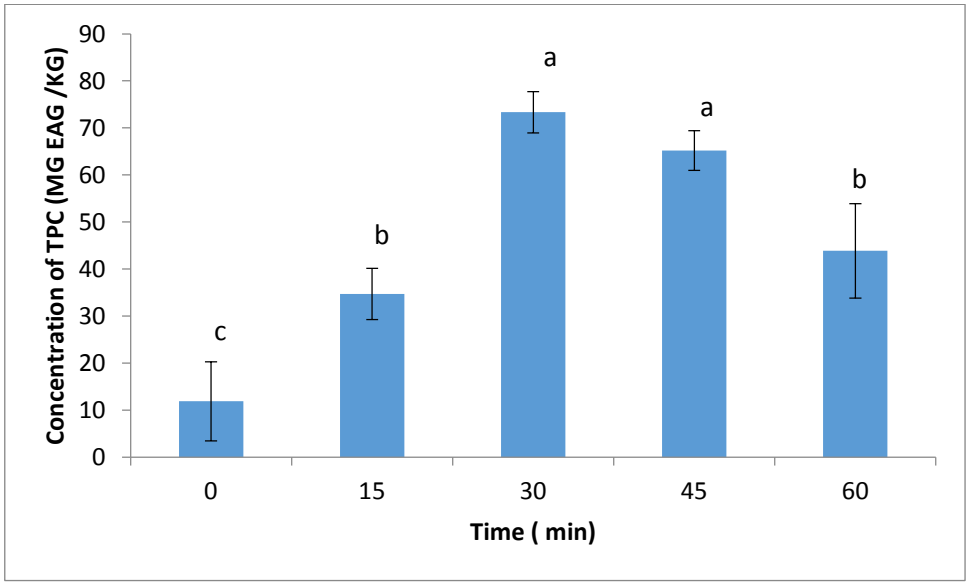


Figure 13: Effect of the irradiation time on TPC content for enriched oil using ultrasound assisted extraction

Values marked by different letters are significantly different (p<0.05)

- **Effect on the carotenoids**

The figure shown a significant increase of carotenoid contents with the increase in the time witch was probably due to which is probably due to the duration of contact of the ultrasound and the vegetal matrix (seeds),the best content with 60 min with a content of (2, 2125 mg/kg).

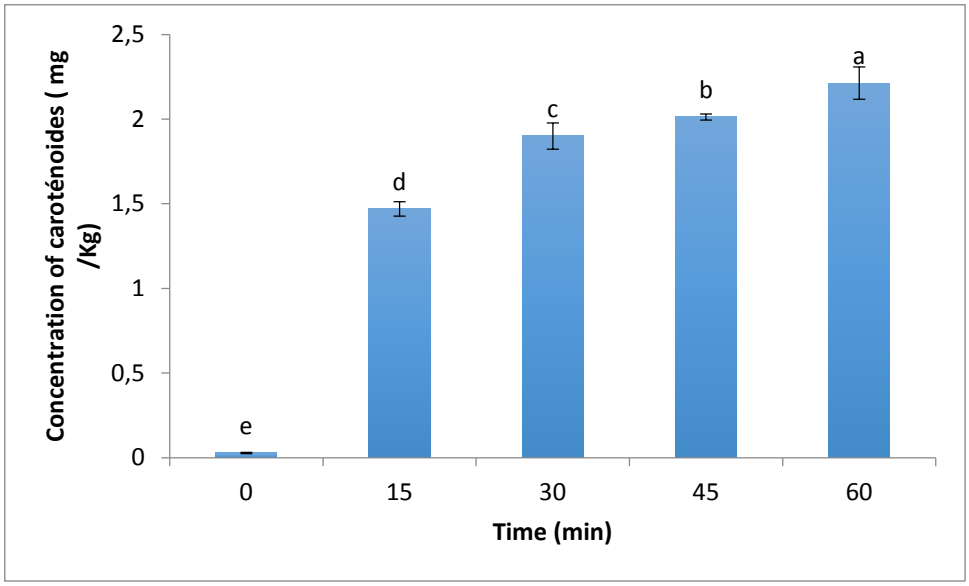


Figure14: Effect of the time on the carotenoids content enriched oil using ultrasound assisted extraction.

Values marked by different letters are significantly different (p<0.05)

- **Effect on the chlorophylls**

The figure shows that the content of chlorophylls increases significantly with the increase in time of oil enrichment which is of (0,26 mg/kg) at (15c°) to (0,58 mg/kg) at (60c°). The time parameter was fixed at (30 min).

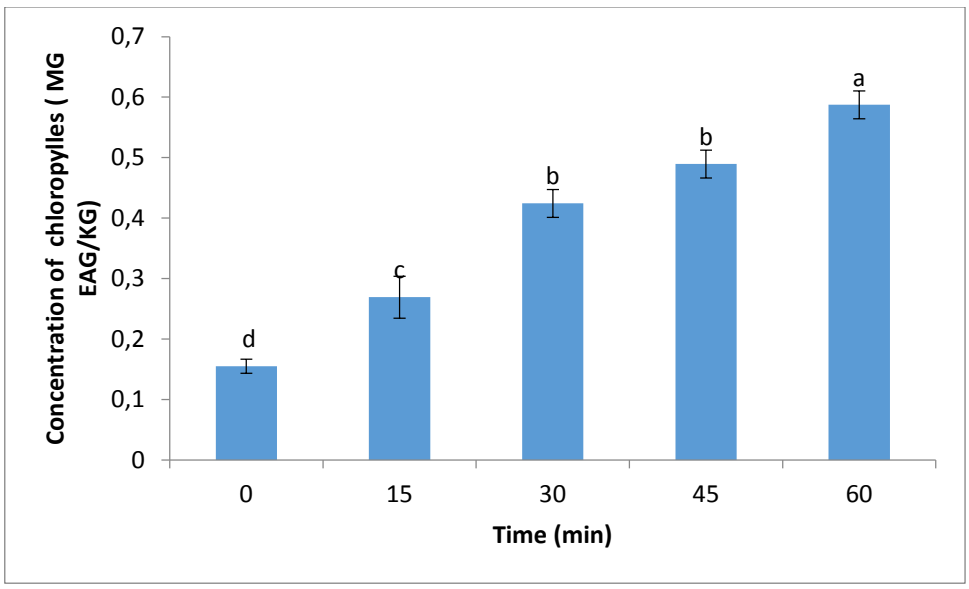


Figure15: Effect of the time on the chlorophyll content of enriched oil using ultrasound assisted extraction

Values marked by different letters are significantly different (p<0.05).

IV.2.3. Effect of powder diameter

After the fixing of time and temperature with the diameter (250), another diameter (125µm) was varied for concluding the best diameter from the extraction. The results of the powder diameter influence on the extraction of the TPC, carotenoids and chloropylls are illustrated in the following figure. The results indicate that the Small diameter powder allows better extraction; This may be due to the increase in the contact surface between the powder and the extraction solvent.

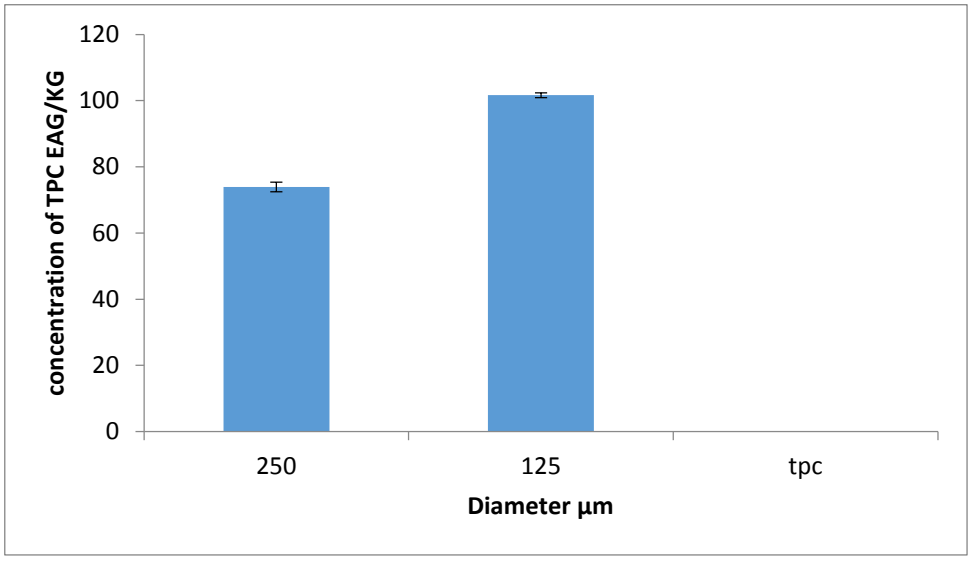


Figure 16: Effect of diameter on concentration of TPC

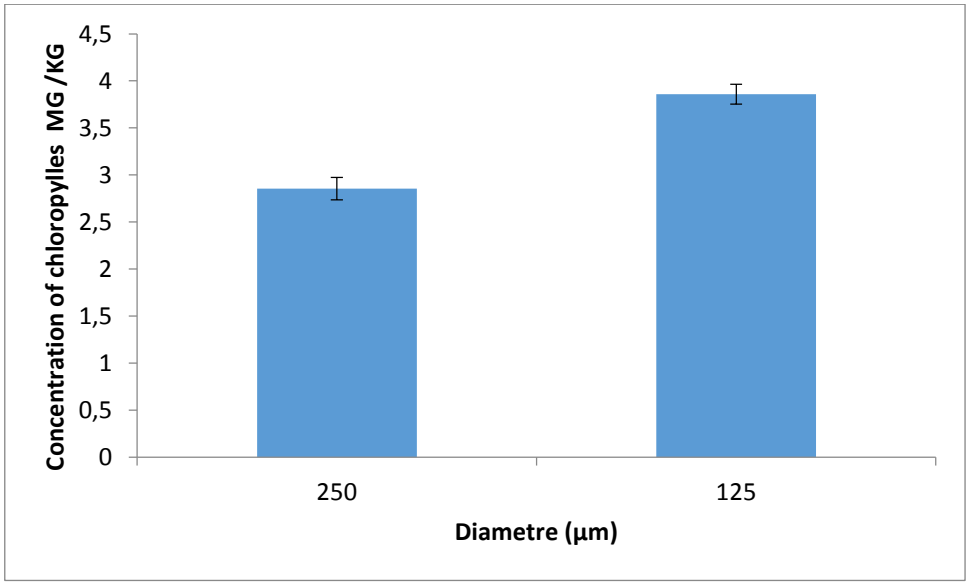


Figure 17: Effect of diameter on concentration of chlorophylls

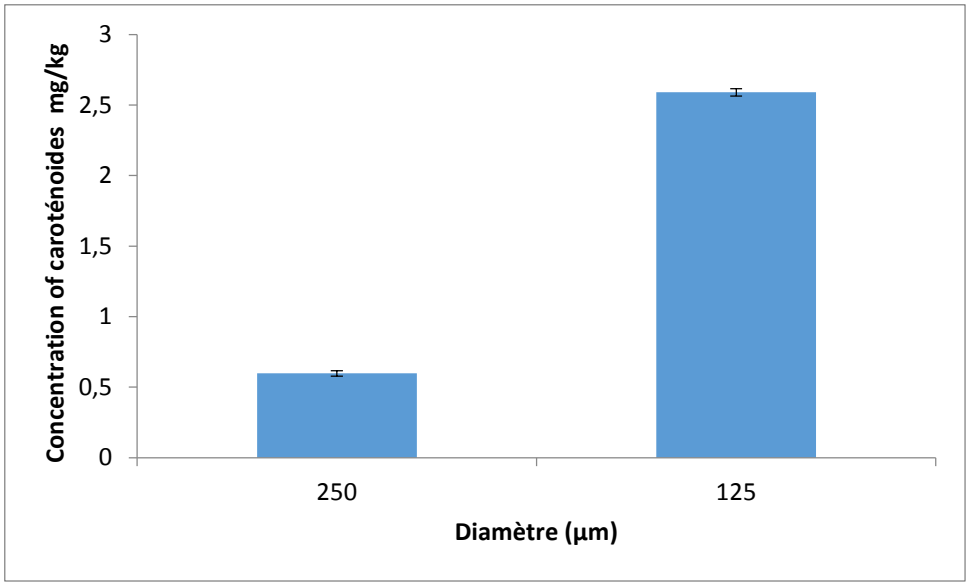


Figure 18: Effect of diameter on concentration of carotenoids

IV.3. Optimization of conditions extraction

IV.3.1. Modeling and fitting the model using response surface methodology (RSM)

The experimental design and corresponding response data for the total phenolic carotenoids and chlorophyll content from enrichment refined oil extract are presented in **(Table 8)**. The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique **(Zhang et al., 2013)** and are given in **(Table8).**

Twelve experimental points run randomly according to the UAE experiment planning,

Table 8: Central composite design with the observed responses values for yield of total phenolic compounds carotenoid and chlorophyll of enriched riffing oil <

Experience	X2 Temperature (C°)	X1 Sonication time (min)	TPC (mg AG/kg oil)	Carrotenoid (mg /kg oil)	Chlorophyll (mg /kg oil)
+-	45	15	99,8518519	2,464	11,9412724
00	30	32,5	114,2493	1,36	1,095
0A	30	50	33,0864	2,065	2,465
00	30	32,5	91,2306	1,665	1,525
00	30	32,5	91,2592	2,07	2,76
0a	30	15	27,65	1,03	1,99
00	30	32,5	80,9628	1,57	1,72
-+	15	50	111,407407	2,67	2,76
a0	15	32,5	142,4198	1,945	0,58
++	45	50	14,8148148	3,91	8,31158238
A0	45	32,5	123,85	4,58	7,18597064
_ _	15	15	28,5432099	1	0,63

The predicted models can be described by the following second-order polynomial equations:

Y_1= 95,32101-7,3096 X_1+0,5446 X_2 - 41,985X_1X_2+35,985X_1^2 – 66,769X_2^2

Y_2= 1, 77170+ 0, 8898 X_1+ 0, 69184 X_2- 0,056 X_1X_2+ 1,279 X_1^2 –0,056 X_2^2

Y_3= 1,51246+3,91147 X_1- 0,17078 X_2-1,2401 X_1X_{2+} 2,8956 $X_1^2{}_+$ 1,4391X_2^2

Where Y is TPC (mg GAE/kg oil). Y_2 is carrotenoid and Y_3 is chlorophyll content. X_1 is the applied temperature (°C) and X_2 is the sonication time (min).The various statistical data such as standard error, sum of squares, F-ratio, or p-value are given in ANOVA **Table(9,10,11) .** The F-ratio in this table is the ratio of the mean-squared error to the pure

error obtained from the replicates at the design center. Values of probability (P)>F less than 0.05 and 0.01 indicate that model terms are significant and highly significant respectively,. The results reveals that the model was highly significant with a p-value < 0.0001, which mean that the model represented the data satisfactorily There is no a large difference between adjR^2 and R^2 **Table** (**9, 10, 11);** indicate that the regression model is a sound one **(Karazhiyan, Razavi, & Phillips, 2011)**.

It can be seen that a quadrature terms of time has the most important influence on TPC contents(P <0,001), followed by the interactions of time and temperature(P=0,0002) than quadratic terms of temperature (P= 0,0042) .Concerning the carotenoid content the dada show that was affected significantly by linear parameters temperature and of the interaction time and temperature (P< 0,0026 , p< 0, 0086) respectively , followed by time parameters at (P< 0,0086)whereas the Quadratic product terms(X_1^2 X_2^2) show no significant effects. The lack of significance of these terms suggests the absence of interactions between variables in the studied zone. The chlorophyll content was nightly affected by the linear parameter of temperature at P<0, 0001 than by the quadratic term of time irradiation at P< 0, 0018 and the cross of time and temperature (P < 0, 0179).

Table 9: Estimated regression coefficients and the analysis of variance (ANOVA) for the experimental results of polyphenol content.

Parameters	**Estimated coefficients**	**DF**	**Sun of squares**	**F ratio**	**Prob > F**
Model			19824,729	0,0765	0,0002*
Intercept	95,329359	1			0,0001*
Linear					
X1-Temperature	-7,308958	1	320,525	3,0051	**0,1337**
X2-Time	0, 543927	1	1,775	0,0166	**0,9016**
Quadratic					
$X1^2$	35, 997773	1	3455,572	32,3978	**0,0013***
$X2^2$	–66,76893	1	11388,775	111,4586	**≤0,001***
Interaction					
X1-X2	- 41,97531	1	7047,706	66,0760	0,0002*
Lack of fit	0,9685	3	45,50358	0,0765	0,9685
Pure error		3	549,460		
R^2	0,968728				
Adjusted R2	0,942669				
RMSE	10, 32766				
CorTotal		11	20464,693		

Table 10: Estimated regression coefficients and the analysis of variance (ANOVA) for the experimental results of carotenoids content

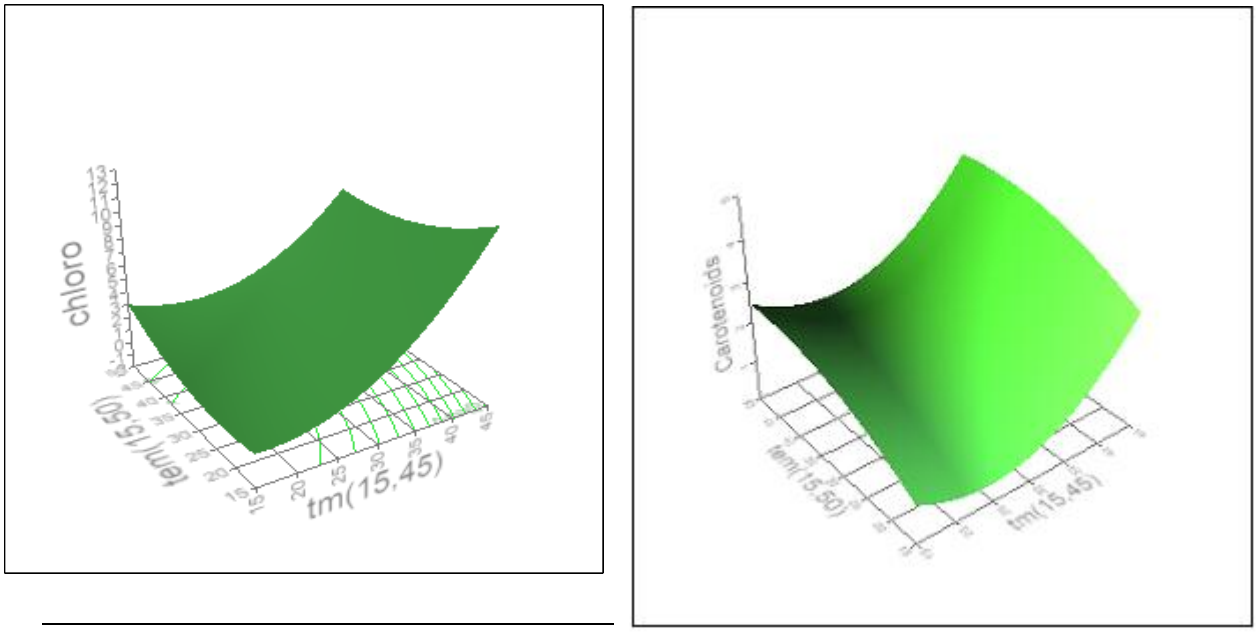
parametrs	**Estimated coefficients**	**DF**	**Sun of squares**	**F ratio**	**Prob> F**
Model		5	12,0035	3,4081	0,0042
Intercept	1, 77170	1			0,0001
Linear					
X1-Temperature	0, 8898	1	4,7508202	24,3038	**0,0026**
X2-Time	0, 69184	1	2,8718002	14,6913	**0,0086**
Quadratic					
$X1^2$	1, 279	1	4,3682134	22,3465	**0,0032**
$X2^2$	–0,056	1	0,5048900	2,5829	0,1591
Interaction					
X1-X2	- 0,056	1	0,0125440	0,0642	0,8085
Lack of fit		3	0,90678	3,4081	0,1704
Pure error		3	0,26601		
R^2	0,910988			0,911099	
Adjusted R2	0,836012			0,836812	
RMSE	0 ,44212				
CorTotal		11	13,176451		

Table 11: Estimated regression coefficients and the analysis of variance (ANOVA) for the experimental results of chlorophylls content

parametrs	Estimated coefficients	DF	Sun of squares	F ratio	Prob> F
Model		5	137,21502	2,1794	0,0002
Intercept	1, 51246	1			0,0001
Linear					
X1-Temperature	2,89559	1	91,797628	115,6329	<0,0001
X2-Time	1,2401142	1	0,174998	0,2204	0,6553
Quadratic					
$X1^2$	2,895599	1	22,3586	28,1641	0,0018
$X2^2$	1,2401142	1	4,1010	5,1659	0,0634
Interaction					
X1-X2	0,2694	1	8 ,293507	10,4469	0,0179
Lack of fit	0,2694	3	3,26506	2,1794	0,2694
Pure error		3	1,4981		
R^2	0,966451				
Adjusted R2	0,938494				
RMSE	0,890994				
CorTotal		11	141,97825		

IV.3.2. Response surface analysis

As expected and according to the response surfaces ,the extraction efficiency in terms of carotenoids et chlorophyll concentrations increases by increasing temperature and time. Thus, the values finally selected correspond to the maximal values chosen to define the experimental domain. After the observations made during the study of the response surfaces (figure 19). Thus the optimization of the temperature and the time is at the maximum of these two parameters at 30 ° C. and 32.5 min for carotenoids and 35 ° C. and 30 min for the chlorophylls **(appendix 5)**



(A) **(B)**

Figure 19: Response surface effect on the carotenoid (B) and chlorophyll (A) yield from; temperature and time effect

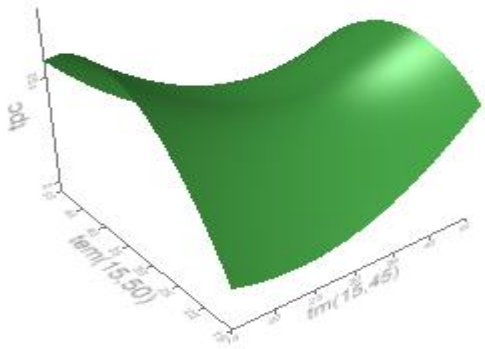


Figure 20: Response surface effect on the TPC (C) yield from; temperature and time effect

IV.3.3. Validation and verification of predictive model

In this present work, RSM method was applied for modeling and optimization of ultrasound-assisted enrichment of reffining oil by seeds *Myrtus communis*. In order to validate the adequacy of the mathematical models, a verification experiment combination factors was carried out under the optimal conditions. The second derivatives were then equaled to 0 and solved in an equation system. The coded values obtained from these equations were thus

Decoded and rounded in order to be applied to the device. The obtained values corresponding to optimal conditions were as follows: Y1= 15 °C, 38 min, Y2=30°C 32,5min and Y3 = 30°C 32, 5min respectively. A repeatability study was conducted by using these optimal conditions to assess the predictive ability of the models Under the optimized conditions, the experimental values for TPC, carotenoids and chlorophylls were (144,09 ±8,04) , (2,09± 0,165 mg/kg oil) (5,19 ± 0,507mg /kg oil) and were very close to the predicted values (145,40 ± 17,8mg EAG/kg oil), (1,77± 0,49 mg /kg oil), (1,51 ±0,99 mg/kg oil) respectively. Predicted values were in close agreement with experimental values and were found to be not significantly different ($p > 0.05$) using a paired test **(Hossain et al., 2012).**

The strong correlation between the real and predicted results confirmed that the response of regression model was adequate to reflect the expected optimization **(Zhanget al., 2013).** the response surface modeling could be applied effectively to predict enrichment of refning oil with Carotenoids chlorophylls and TPC from Myrtle seeds by product. **Appendix(5)**

IV.4.Optimal conditions

IV.4.1. Comparison between the refining and enriched refining oil:

The optimal conditions retained in this study are 15 C° and 38,5Min for temperature and the time respectively. In order to see whether the model suggested is valid to represent the studied answers, a comparison was made with the experimental values carried out under these optimal conditions **(table 12).** The results show that the predicted contents are very close to the experimental contents for the 3 studied answers. That means that this model is suitable and valid to represent the study carried out

Table 12: Optimum conditions for enrichment assisted by bath ultrasounds of oil refined by seed of *Myrtus Communis*

	Valeur prédite	Valeur expérimentale
TPC (mg EAG Kg-1)	145, 40± 17,78	144.09 ± 8,04
Caroténoïdes (mg Kg $^{-1}$)	1,77±0,49	2,09166±0,165
Chlorophylles (mg Kg $^{-1}$)	1,51 ± 0.99	5.19±0.507

IV.4.2. Reducing power:

The reduction is a test which makes it possible to highlight the capacity donor of electron. Moreover, the reducing property of a compound can be useful like a significant indicator of its antioxidant activity potential **(Jeong and Al, 2004; Kumaran and Al, 2007).**

Reduction of the extracts of the species. is probably due to the presence of grouping hydroxyl in the phenolic compounds which can be used like donor as electron. Consequently, the antioxydants are regarded as reducers and inactivateurs of the oxidants **(Siddhuraju and Becker, 2007**). In the same way Some former studies also showed as the reduction of a compound can be useful like a significant indicator of its antioxydant activity potential (**Jeong and al, 2004; Kumaran and Al, 2007).**

According to the (**figure 20**) we notices that the reduction of enriched oil refined is higher than that of not enriched oil refined.

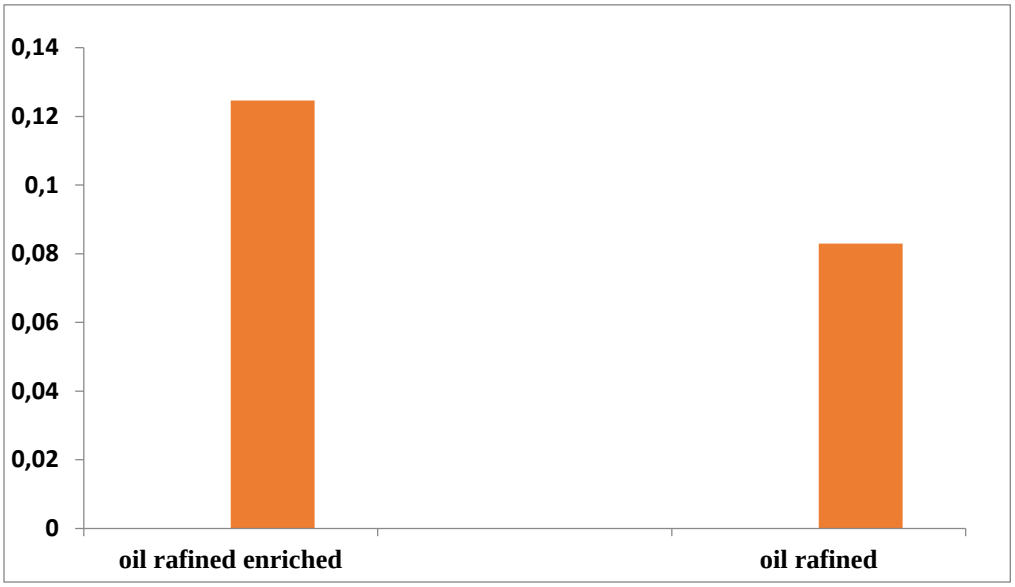


Figure 21: Reducing power of refined oil enriched and not enriched oil

IV.4.3. Scavenger activity on the radical 2,2-di phényl-1-picrylhydrazyle (DPPH)

The trapping of the stable radical of DPPH is a method employed to evaluate the antioxidant activities. This method characterized by **(Dudonne et al. 2009)** and the results obtained for oils refined and enriched expressed in % by inhibition of DPPH are summarized in the (**figure 21**) (antioxydant capacity of the extracts methanolic of oils analyzed).

This purple chromogene is easy to use, has a great sensitivity, allows the fast analysis of the antioxidant activity of a great number of samples and gives reproducible results (**Gulçin and Al, 2010).**

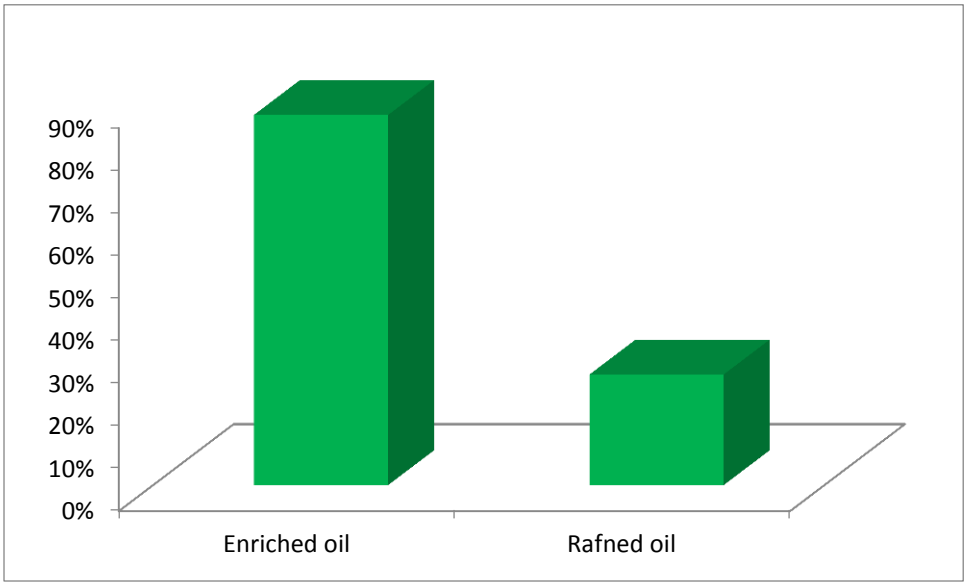


Figure 22: anti capacity radical off extracted methanolic off oil analyzes

According to the results obtained, the results of the capacity antiradicalaire of the extracts of oils (refined and refined enriched) indicates that the capacity to trap radical DPPH by enriched oil refined is more important (78 ± 0,15%) three times higher than that of not enriched oil refined (26 ± 0,015%).

We can say that the whole of the recorded results, the optimal conditions suggested by the experimental design, that the transfer of polyphenols of seeds of myrtle in oil refined could be place the in charge of this antioxydant activity.This antioxydant activity present in the extract methanolic makes it possible to inhibit lipidic oxidation.

These activities could be related to the richness of the polyphenol extracts. Because according to (**Turkmen and Al 2007),** the polyphenols seem to be effective hydrogen givers to radical DPPH, because of their ideal structural chemistry. The others minor phenolic compounds should not be neglected, by what synergy between the various chemicals should be taken into account in the biological activity **(Bourgou and Al, 2008).**

Conclusion

Conclusion

This study aims to extract the phenolic compounds of under products of the myrtle (seed of *myrtus communis*) assisted by bath ultrasounds and optimization the conditions of extraction in order to work out processes of simple and effective extraction to employ them with fine industrialists and analytical.

A plan of Box and Benken, was set up to study the effect of two variables independent time (min) and temperature (c°) on the extraction of the total phenolic compounds,carotenoids ,and chlorophylls in order to define the model mathematical allowing the optimization of the conditions of extraction.

A preliminary study has makes it possible to determine the tops and bottoms for each studied factor.

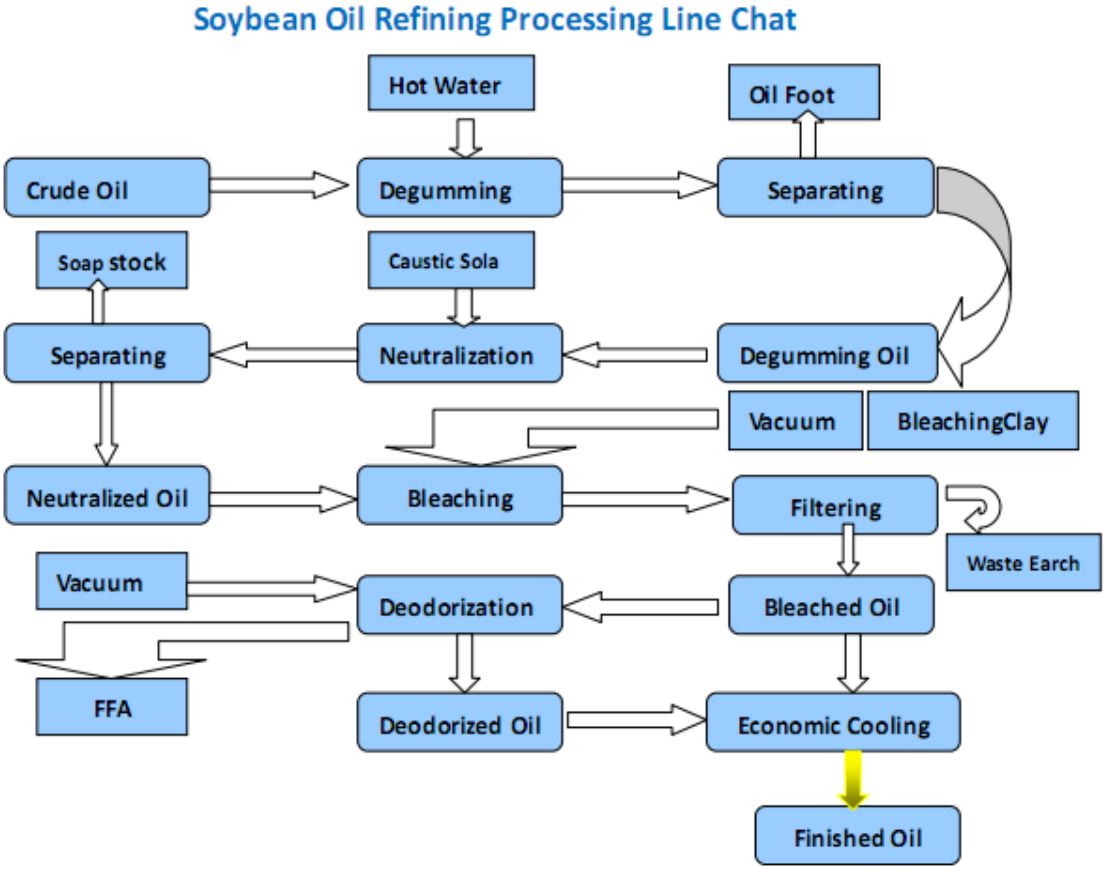
the variance analysis for the effect of the factors onTPC,carotenoids and chloropylls in the case of extraction by bath ultrasounds, gives coeffecients of R22 determination of 0,97;0,91 and 0,97 respectively and the values of the coeffecients of determination adjusted (R adjusted) are about 0,94 ,0,83 and 0,93 this analyze watch that the model is significant <0,0002.

In conclusion this modest work, constitutes at the same time a valorization of under produced products of myrtle (seed of *myrtus communis*) which is rich in phenolic compounds having antioxidant properties and in addition an improvement of the quality of oil refined while bringing more active ingredients to him.

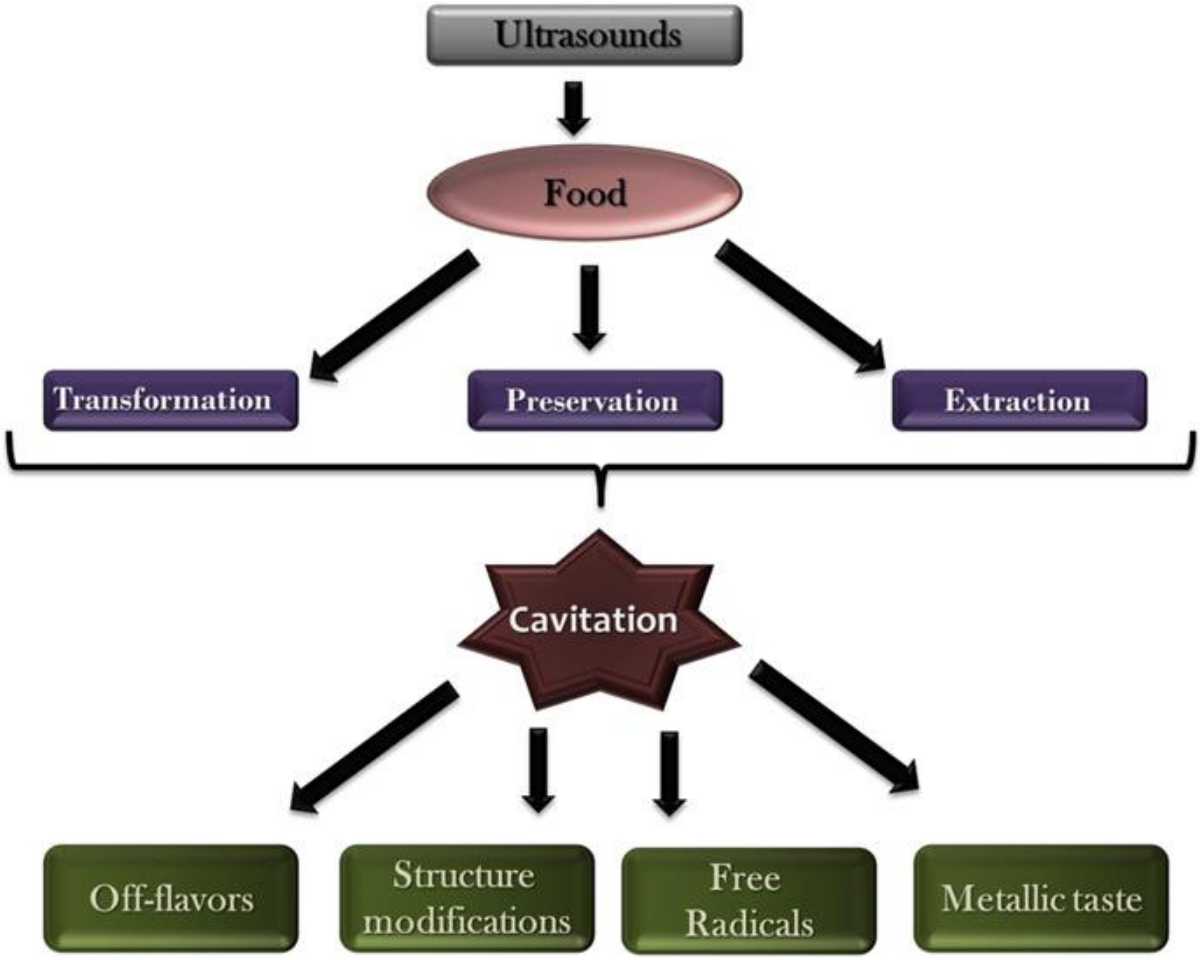
The present study is certainly incomplete, but remains an open door for other work, for that it would be interesting of:

- An optimization of another parameters influencing the rate of extraction of the TPC (solvent of extraction, amplitude)
- To characterize the TPC by a chromatography in high performance liquid phase coupled with the mass spectrometry (HPLC-MS).
- A study extended to other parts of the plant
- To evaluate the effect of heating on enriched oil (test of crackling)
- Study of the deterioration of oils (proportioning of the products of oxidation)
- Comparison of the conventional method by maceration with new technologies.

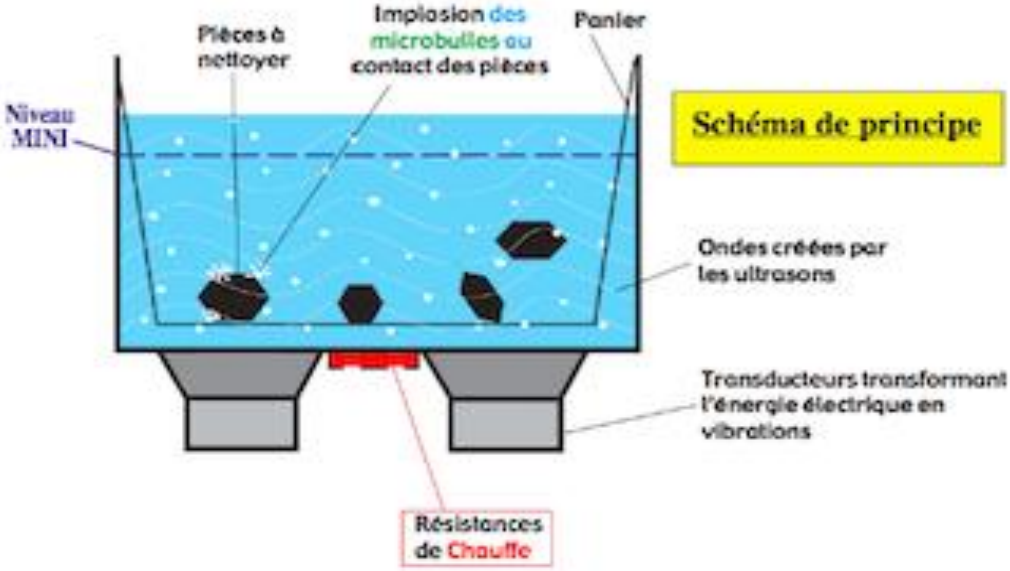
Appendix



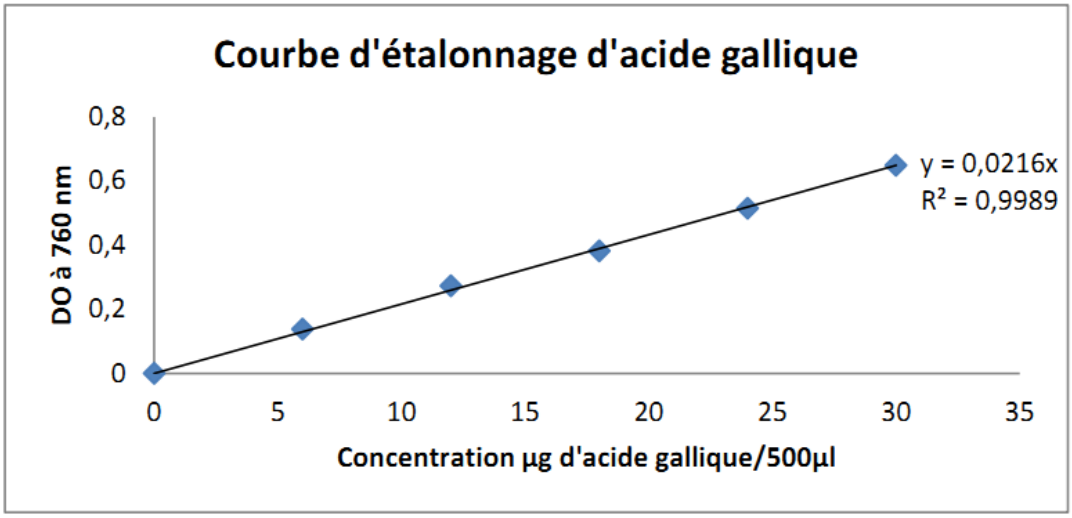
Appendix 2: Chemical effects generated by cavitation phenomena



Appendix 4: Principe of bath ultrasound

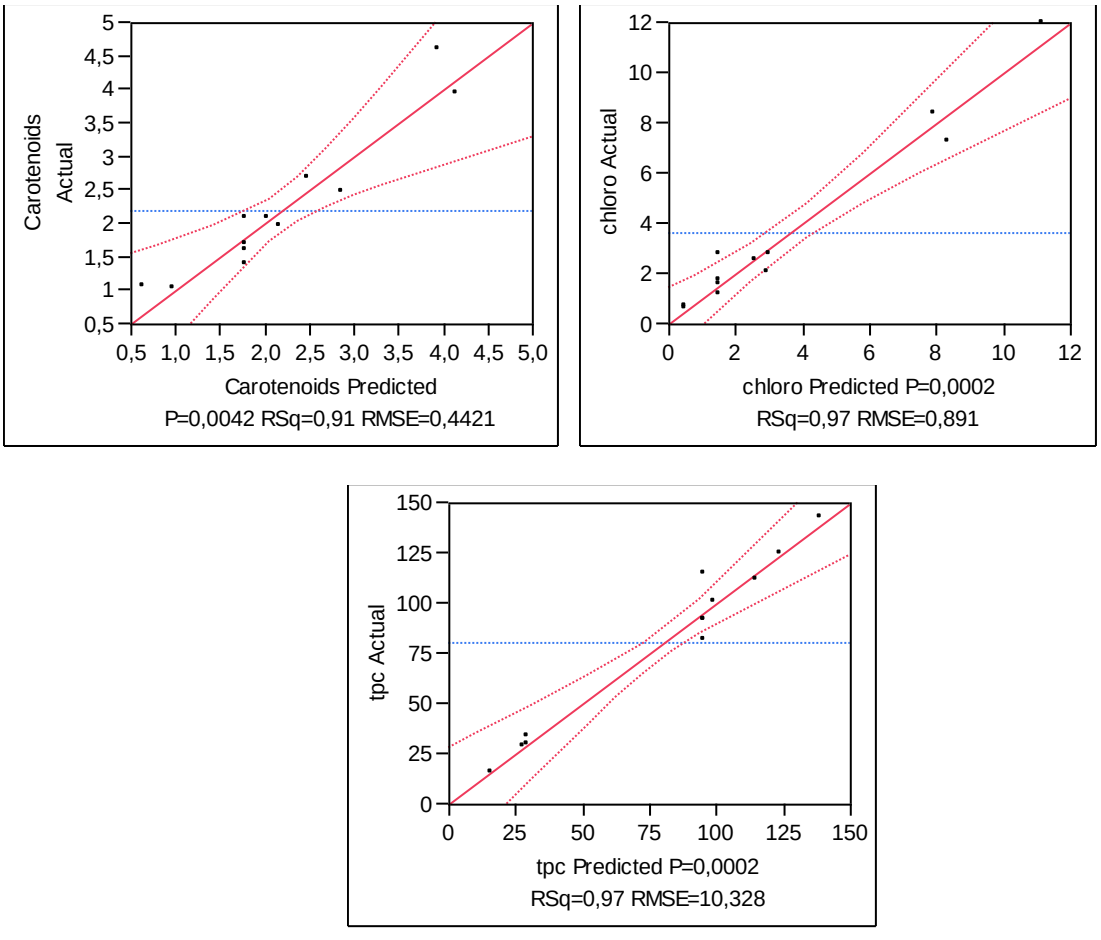


Appendix 4: Courbe d'étalonnage de l'acide gallique



Courbe d'étalonnage de l'acide gallique.

Appendix 5: Results actual by predicted plot



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Abstract

In this study, an ultrasonic bath extraction method was used for the extraction of total polyphenols from myrtle byproducts (*Myrtus Communis* seed).

The possibility of improving the nutritional value of refined oil by enriching phenolic compounds with *Myrtus Communis* seeds. An application of experimental plans is envisaged in order to optimize extraction conditions; the effect of independent variables (Time, temperature) on the TPC , caroteinoides and chlorophylls contents was evaluated using the RMS surface area methodology.

Analysis of the variance confirmed that the contribution of a quadratic model and the effect of interactions are significant for the combination response were a temperature 15°C and a extraction time 38.5min in other the DPPH test performed and reduced power, confirmed its enrichment in antioxidants.

Key Word: Optimization, experimental design, surface response, enrichment, ultrasonic bath, phenolic compounds.

Résumé

Dans cette étude, une méthode d'extraction par bain ultrasons a été utilisée pour l'extraction des polyphénols totaux à partir des sous-produits de myrte (graine de *Myrtus Communis*).

La possibilité d'améliorer la valeur nutritionnelle de l'huile raffiné en enrichissant par les composés phénoliques des graines de *Myrtus communis* une application des plans d'expériences est envisagée afin d'optimiser les conditions d'extraction, l'effet des variables indépendantes (temps, température) sur les teneurs en TPC ,carotenoids et chlorophylls a été évaluer en utilisant la méthodologie de surface de réponses RMS.

L'analyse de la variance a confirmé que la contribution d'un modèle quadratiques et l'effet d'interactions sont significatifs pour la réponse de combinaison étaient une température 15c° et un temps d'extraction 38,5 min en autre le test de DPPH réalisé et le pouvoir réducteur, a confirmé son enrichissement en antioxydants.

Mots clés : optimisation, plan d'expérience, réponse de surface, *Myrtus Communis*, enrichissement, bain ultra-sons, composés phénoliques.