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MASTER

Enrichissement des huiles végétales par des antioxydants naturels

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Table of contents

List of abbreviations

ANOVA: Analysis Of Variance

BBD: Box Benken Design

CM: conventional maceration

DM: dry matter

DPPH: 2, 2- Diphenyl – picrylhydrazyl

FC: Folin Ciocalteu reagent

GAE: Gallic Acid Equivalent

OFI: Opuntia Ficus Indica

OO: olive oil

RP: Reducing Power

RSM: Response Surface Methodology

RO : refined oil

TFC: Total Flavonoids Content

TP: Total polyphenols

TPC: Total Phenolic Content

UAM : ultrasound assisted maceration

UV: UV visible

V/V: volume/ volume

WHO: World Health Organization

W/V: weight/ volume

List of table

List of figure

Introduction

Introduction

The inhabitants of Mediterranean countries have a longer life expectancy and lower risk of chronic diseases. Epidemiological studies indeed suggest that the diet and lifestyle of these populations lead to decreased rates of cancer, diabetes and cardiovascular diseases (**Achat; 2013)**. Olive oil is typically the main lipid source in this region, being used for cooking, cosmetics and medical purposes. The nutritional and health effects of olive oil has been mainly assigned to an adequate fatty acid profile (98% of total oil weight) and to its content in secondary metabolites (2% of total oil weight) namely phenolic compounds **(Ben Hassine et *al*; 2007).**

Cactus pears and laurel are abundant plant in the Mediterranean regions, provide a rich source of phenolic compounds, essential oil rich of aroma and very important antioxidant molecules **(Conforti et *al*; 2006, Jovic et al; 2018)**, which may act by different mechanisms (antioxidant, signaling) to protect against free radical attacks **(Achat et al; 2012)**. Moreover, cactus pears generate a large amount of by-products (seeds and peels) in food transformation and can be used as a cheap source of high added-value phenolic compounds. As well as the aromatic plant laurel (leaves)

A large part of current research aims to enrich vegetable oils with antioxidants in order to improve their organoleptic quality, their composition and especially their stability and antioxidant activity **(Bouaziz et *al*; 2008, De Leonardie & Macciola; 2012, Jovic et al; 2018)** Conventional maceration could be organic solvent extraction prior to addition to food matrix or steam distillation of essential oil. Both procedures are either too long or demand too high temperature which causes a damage of the plant material. Ultrasound-assisted maceration (UAM) is one of the novel procedures for the extraction of beneficial content in aromatic and no aromatic plants (**Jovic et al; 2018)**

The current study propose a direct enrichment of edibles oils namely olive oils with plant material (cactus peels and laurel leaves). Due to the many factors that influence the UAM, 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

UAM process parameters (temperature, extraction time and ratio) by employing a Box–Behnken design to maximize extraction of antioxidants from cactus pears and laurel leave. After then a comparison was carried out between UAE and conventional method.

In order to better situate the context of this research, a bibliography was presented on olive oil, vegetable matrices (prickly pear and laurel), antioxidant and the enrichment of edible oils.

Bibliography

I. Olive oil

The olive tree is an exceptional tree that operates a real fascination, a symbol of peace and durability thanks to a longevity out of the ordinary; it has been part of the life of Mediterranean civilizations for a long time. It has always spread to many essential daily needs including the oil produced from fruit **(Vincent-Baudry et *al*., 2005).**

Olive oil is a very interesting product from a nutritional point of view. This is primarily due to its fatty acid composition, sensitive to the phenomenon of oxidation. In addition to its particular composition in fatty acid, olive oil is especially interesting for its minor compounds including phenolic compounds or polyphenols. The nutritional value of these active substances lies in their strong antioxidant capacity, which could have a major role in improving the stability of olive oil **(Petrucciol et *al*., 1988).**

I.1. Definition:

According to official standards, olive oil can be obtained only from the fruit of the olive tree (*Olea europaea* L) and only by the use of physical processes. The absence of a refining step allows the olive oil to retain all its antioxidants, because they will not be eliminated during this process (**Veillet., 2010).**

I.2. Extraction technology

The processing of olives for the extraction of the oil is done by purely mechanical processes (by pressure or centrifugation). There are generally three olive oil extraction systems.

I.2.1. Discontinuous system of extraction by press

This system uses metal screw presses or, if necessary, hydraulic presses. It allows obtaining a non-pungent oil and rich in polyphenols (**Chimi., 2006, Salomone et *al*., 2015**).The operations of grinding and pressing the olive paste, conducted in the open air, can cause the alteration of the oils. An auto oxidation of the oil is triggered by the presence of air causing degradation of unsaturated fatty acids and then the formation of hydroperoxides which decompose giving rise to volatile products leading to a state of rancidity of the oil. This system also generates significant amounts of vegetable water (60 to 70 L per 100 Kg of olives**) (Ben Hassine et *al*., 2007).**

I.2.2. Continuous system with two-phase centrifugation

The olive oil extraction technology works with a new decanter with two-phase centrifugation (oil and pomace), that does not require the addition of water for the oily and solid phase separation containing the olive and vegetable oils. Among its advantages, this system

generates a slightly higher oil yield than the others. It provides an oil rich in total polyphenols which makes it more stable. This system is more respectful of the environment because it does not increase the volume of liquid effluent (waterweed). (**Ben Hassine et *al*., 2007).**

I.2.3. Continuous system with three-phase centrifugation

The three phases are oil, vegetable water and pomace. The introduction of these "continuous" installations has made it possible to reduce processing costs and the storage time of olives, resulting in olive production of lower acidity.

Nevertheless, the high inputs of hot water (40 to 60% of the weight of the dough), make the oil extracted is depleted in aromatic and phenolic compounds. These compounds pass partially in the vegetable waters. This system also gives rise to pomps with high levels of moisture (45 to 55%) **(Ben Hassine et *al*., 2007).**

I.3. Chemical composition of olive oil

The chemical composition of olive oil depends largely on the variety of fruit, agronomic conditions and degree of maturity, extraction processes and storage conditions **(Dugo et *al*., 2004).** Like all vegetable oils, olive oil contains major and minor elements, it consists of a saponifiable fraction and an unsaponifiable fraction **(C.O.I., 2001).**

I.3.1. Saponifiable fraction

It accounts for most (99%) of the overall composition of olive oil. It is formed mainly of triglycerides and fatty acids.

a. Glycerides

These are esters of fatty acids and glycerol. Glycerides are the main component of olive oil, about 98% (**Ollivier et *al*; 2004**). The main triglycerides of olive oil are triolein (40 to 60%), dioleopalmitin (10 to 20%), dioleolinoline (10 to 20%), palmitoololinolin (5 to 7%) and the Dioleostearin (3-7%) (**Ryan et *al*., 1998, Boskou et *al*., 2006).**

b. Fatty acids

Fatty acids are responsible for the sour taste and the pronounced odor of the oil. They are present either in the saturated state (12.6 to 19.7%), or monosaturated. Table I summarizes the composition of olive oil in fatty acid **(C.O.I., 2001).**

Table I: Fatty acid composition of olive oil by gas chromatography **(C.O.I., 2001).**

Fatty acids	**Symbol**	**Percentage (%)**
Oleic acid	C18 :1	55 – 83
Linoleic acid	C18 :2	3,5 – 21
Palmitic acid	C16:0	7,5 – 20
Stearic acid	C18:0	0,5 – 5
Palmitoleic acid	C16 :1	0,30 - 3,5
Linolenic acid	C18 :3	≤ 1
Arachidic acid	C20:0	≤ 0,6
Gadoleic (eïcosenoïque) acid	C20 :1	≤0,4
Myristic acid	C14:0	≤0,05
Heptadecanoïque acid	C17:0	≤0,3
Heptadecenoïque acid	C17 :1	≤0,3
Heptadecenoïque acid	C22:0	≤0,2
Lignoceric acid	C24:0	≤0,2

I.3.2. Unsaponifiable fraction

The unsaponifiable fraction or non-triglyceride is often accompanied by the term "minor components". It consists of hydrocarbons, sterols, terpene alcohols, tocopherols, phenolic compounds and pigments (chlorophyll, carotenoids) (**Jacotot., 1993).**

a. Phenolic compounds

One of the most important characteristics of olive oil is its content of phenolic compounds (hydroxytyrosol and tyrosol) with antioxidant properties. They include a variety of different substances that make olive oil taste so different from other oils and contribute to its good stability by increasing its resistance to auto-oxidation at the cellular level. Their content depends on the degree of maturity of the olives **(Rovellini-Cortesi., 2003)**, the season and climatic conditions (**Salvador et *al*., 2003**), the health status of the olives, the variety and oil extraction system (**Gimeno et *al*., 2002**). These molecules strongly contribute to the pungent taste, astringency and bitterness of oils (**Haddam et *al*., 2014).**

b. Tocopherols

The total content of tocopherols in olive oils is very variable since it has been reported in a range from a few mg to 450 mg / kg of oil **(Boskou et *al*., 2006).**

c. Hydrocarbons

The main hydrocarbon of olive oil is squalene ($C_{30}H_{50}$). It appears in the pathway of cholesterol biosynthesis. Extra virgin olive oil contains squalene at about 400 - 450 mg / 100g, while refined olive oil contains 25% of less (**Owen et *al*., 2000**). Squalene has a protective effect at low temperatures and in the dark **(Velasco & Dobarganes., 2002).**

d. Sterols

The plant sterols called phytosterols occupy the largest part and represent the major constituent of the unsaponifiable matter of oils (**Leroy., 2001**), non-glyceric constituents, they represent by weight about 50% of the unsaponifiable. The sterol composition is specific for each plant species. Among the factors that influence this content are the variety of olives and their degree of maturity (**Gutierrez et *al*., 1999**). The total amount of sterols in the extra virgin olive oil varies from 113 to 265mg / 100g, the main sterol of which is ß-sitosterol, which represents up to 75-90% of the total, delta-5 avenasterol (3 to 14%), campesterol (2 to 4%), stigmasterol (1 to 2%) and cholesterol (<0, 3) (**Velasco & Dobarganes., 2002).**

e. Aromatic compounds

They are responsible for the delicate aroma of olive oil, they consist of a mixture of volatile compounds: aldehydes, alcohols, esters, hydrocarbons, ketones (**Boskou et *al*., *2006 a*, Kalua et *al*., 2007**). Its content is influenced by the cultivar and is highly dependent on the activity of enzymes in the lipoxygenase pathway (**Dhifi et *al*., 2005, Runcio et *al*., 2008**).

f. Pigments

- Chlorophylls: With a level of 1 to 20 mg / kg (40 to 80% are pheophytins) (**Ranalli., 1992**), chlorophylls are present in fresh olive oil. Chlorophylls a and b are easily degraded to pheophytins (brown in color). The latter and chlorophylls are mainly responsible for the characteristic color of olive oil (**Rahmani., 1989, Gandul-Rojas., 1996).**

- Carotenoids: The carotenoid pigment most found in olive oil is β carotene (Provitamin A) which has a vitamin and antioxidant action. Its level varies from 0,3 to 3,7 mg / kg of oil and 2 mg of β-carotene are converted into 1 mg of vitamin A. Provitamin A is converted into vitamin A during intestinal absorption (**Kataja- Tuomola., 2008).** Some authors have noted that biological and technological factors; the extraction system, the mode and shelf life and

particularly the ripening of the fruit influence the carotenoid pigment composition of olive oil (**Nieves - Criado et *al*., 2008).**

II. Vegetable matrix

II.1. Cactus

II.1.1. Origin and classification

Opuntia ficus-indica, (OFI) commonly called prickly pear or nopal cactus, belongs to the *Cactaceae* family, that includes about 1500 species of cactus. The *Opuntia* kind is originating in Mexico **(Orwa et *al*., 2009).** OFI is a tropical and subtropical plant. It can grow in arid and semi-arid climates with a geographical distribution encompassing Mexico, Latin America, South Africa and Mediterranean countries (**Butera et *al*., 2002)**. It was introduced initially in Spain and later at the 16th century in the North and the South of Africa. **(Le Houerou., 1996, Halmi et *al*., 2015).**

Classifications of cactus pears is: **(Halmi et *al*., 2015).**

Kingdom: *Plantae*

Under-kingdom: *Tracheobionta*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Under-class: *Caryophyllidae*

Order: *Caryophyllales*

Family: *Cactaceae*

Genus: *Opuntia*

Species: *Opuntia ficus indica*

II.1.2. Composition

The prickly pear is a succulent fruit, not very acid and rich in sugars; what returns it delicious and soft **(Kaanane., 2000, Piga., 2004, Feugang et *al*., 2006)**. The average composition of prickly pear is summarized in the following table:

Table II: Composition of prickly pear (**Piga., 2004**).

Parameters	**Value**	**Parameters**	**Value**
Pulp (%)	43-57	Mg (mg/100g)	16.1-98.4
Seeds (%)	2-10	Na (mg/100g)	0.6-1.1
Peeling (%)	33-55	K (mg/100g)	90-217
pH	5.3-7.1	P (mg/100g)	15-32.8

Acidity (% citric ac)	0.05-0.18	Proline (mg/L)	1768.7
Water (%)	84-90	Glutamine (mg/L)	574.6
Proteins (%)	0.2 – 1.6	Taurine (mg/L)	572.1
Lipids (%)	0.09-0.7	Serine (mg/L)	217.5
Fibers (%)	0.02-3.1	Alanine (mg/L)	96.6
Total sugars (%)	10-17	Glutamic acid (mg/L)	83.0
Vitamin C (mg/100g)	1-41	Méthionine (mg/L)	76.9
Calcium (mg/100g)	12.8-59	Lysin (mg/L)	53.3

II.1.3. Compartments of prickly pear fruit

a- The peels

Peels of OFI represent a large proportion of the whole fruit (from 40% to 50%) and constitute a source of bioactive compounds, notably phenolics, flavonoids and betalains (**Arrizon et *al*., 2006, Kuti., 2004).** It is delivered in a bow in sky of active color of the green, yellow, orange, red, purple and same to the brown **(Feugang et *al*., 2006)**. Peels are a new dessert of a dietary fiber and its content in galacturonic acid was superior to that of commercial cladode cactus racket **(El-Salid et *al*., 2011).**

b- Pulpy juice

Juice production is one of the most frequently utilized fruit and vegetable technology **(Tesoriere et *al*., 2005).** In some countries, cactus pear juice is consumed at home, in vegetarian restaurants or in local health-food stores. Since technological problems are associated with its production, no commercial products are produced at industrial level **(Cassano et *al*., 2010).**

c- Seeds

The prickly pear seeds are characterized by their hardness due to the presence of fibers hard and forms punts, at least reinforces or lenticular. The percentage and the number of seeds per fruit vary according to several factors of which variety, the physiology and environment of culture (**Habibi., 2004, Reyes-Aguero et *al*., 2005).** Seeds contained in the pulp, accounts for 2 to 10%. Studies have been reported the chemical composition of seeds oil of OFI: a high degree of unsaturation where linoleic acid is the major fatty acid (56.1–7: 7%), rich in oleic (C18:1) acids (16.7%); 87% of the total fatty acids. **(Ghazi et *al*., 2013).**

II.1.4. Importance

- The first economic importance of this plant relies on the production of edible fruits. It is mainly consumed fresh or converted into drinks (nectars, juice), jams or marmelades. This food transformation generates a large amount of by-products (seeds and peels) (**Habibi., 2004**).

- The plant genus OFI has proved to be an important source of therapeutic agents, because of the diversity of pharmacological properties and chemical structures. OFI has been used in the traditional medicine of diverse countries for a long time (**Park et *al.*, 2001, Ammar et *al.*, 2018).**

II.2. Laurel (*Laurus nobilis*)

II.2.1.Origin and classification

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (**Nostro et *al., 2000***). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (**Pierangeli et *al.*, 2009**).

- Laurel (*Laurus nobilis*) is an evergreen tree cultivated in many warm regions of the world, particularly in the Mediterranean countries (Turkey, Greece, Spain, Portugal, Morocco) and Mexico. (**Barla et *al.*, 2007**). The Lauraceae comprise 32 genera and about 2.000-2.500 species.

- Taxonomic classification of laurel is**:**

Reign: *Plantae*

Under-reign: *Tracheophyta = Tracheobionta (Cormophyta)*

Division : *Magnoliophyta*

Class : *Magnoliopsida*

Order : *Laurales*

Family: *Lauraceae*

Genus: *Laurus*

Species: *Laurus nobilis L* **(Guedouari., 2012)**

II.2.2. Morphological description

The noble laurel is a generally dioecious tree (with separate sex), 2 to 10m in height sometimes reaching a height of 15-20 m 20 to 30 feet) with branches pointing upward, and always green that grows wild or cultivated **(Guedouari., 2012).** Leaves are evergreen, alternate,

elongated to lanceolate, about 10 cm long and 3 to 5 cm wide; they terminate at the tip of both sides and are short stalked; their lamina is glabrous, entire, often slightly wavy and thickened at the edges, curved inward, of a dark green. **(Guedouari., 2012).** The flowers are small; fragrant star-shaped in late spring, whitish to yellow in color, flowering takes place from March to May. Fruits or bay berries (globular berries, fleshy aromatic drupe) resemble a small olive with oval or ellipsoid shape **(Guedouari., 2012).**

- Laurel leaves were known to be a valuable source of mucilages, vitamins, and antioxidant molecules (polyphenols: flavonoid and tanins. **(Guedouari., 2012).**

- The leaves of this plant have been used to treat epilepsy (**Aqili-khorasani., 1992, Zargari., 1990)**, neuralgia and Parkinsonism (**Aqili-khorasani., 1992).** The essential oil obtained from the leaves of this plant has been used for relieving hemorrhoid and rheumatic pains (**Zargari., 1990**). Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (**Buchbauer., 2000).**

III. Antioxidants and enrichment of oils

III.1. Antioxidants

The antioxidant is a substance, which inhibits or delays significantly the oxidation of a substrate, whereas it is presented in very weak concentration in the medium where it intervenes (**Sies., 1993).** Plants constitute very important sources of antioxidants. The natural antioxidants whose efficacy is more recognized in agri-food as well as for the human health are tocopherols, carotenoids and polyphenols. (**Sebei et *al*., 2007)**

III.1.1. Tocopherols

They are food antioxidants, but especially their physiological role in humans, as protectors of membrane structures and lipoproteins or to fight against the oxidative stress. They act either as electrons donors , delaying the reactions of oxidations, or as electron acceptors acting on singlet oxygen, thus inhibiting oxidations induced by this last, prevents the appearance of hydroperoxides by trapping radicals LOO• **(Sebei et *al*., 2007).** Thus, vitamin E has the capacity to capture and destabilize (by resonance) the single electron of the free radicals according to the reaction

tocopherol-OH + LOO•→ tocopherol-O•+ LOOH

(LOO•: lipidic free radical).

Vitamin E is often used as food conservative (E306 with E309) to prevent food rancidity by the free radicals (**Choe et *al*., 2009**).

Tocopherols protect the polyunsaturated fatty acids against natural oxidation. A tocopherol molecule can protect 10^3 with 10^6 molecules from polyunsaturated fatty-acids.

The antioxidant activity of tocopherols mainly based on the existence of the tocopherol-tocopherylquinone reduction system. Indeed, a molecule of tocophérols can reduce two lipidic radicals by forming a molecule of the α-tocopherylquinone (**Sebei et *al*., 2007)**

III.1.2. Carotenoids

Carotenoids are pigments, their antioxidant activity is linked to their long polyene chain which allows them to react with the radicals ROO •, HO •, O 2 • -, R • by simple electrophilic addition and electron transfer. Allow, in particular, neutralizing singlet oxygen (**Valko et *al*., 2006**).

Car + ROO•+ H + → Car•+ + ROOH

Car-H + ROO•→ Car•+ ROOH

Car + ROO•→ • Car-OOR

Car: β-carotene

Figure 1: Mechanism reflecting the antioxidant activity of carotenoids, case of the ROO• (**Valko et *al*., 2006**).

III.1.3. Phenolic compounds

Polyphenols, are specific molecules of the vegetable kingdom and belong to their secondary metabolism. They are found in plants, from roots to fruits. Their functions are not strictly essential for the life of the plant; however, these substances play an important role in the interactions of the plant with its environment, thus contributing to the survival of the organism in its ecosystem. The term "phenol" roughly includes 10000 identified natural compounds. The fundamental structural element which characterizes them is the presence of at least a phenolic nucleus with 6 carbons, to which at least one free hydroxyl (OH) group is bonded directly or engaged in another function: ether, ester or glycoside (**Achat et *al*., 2012).**

The following table presents the principal classes of the phenolic compounds.

Table III: Principal classes of the phenolic compounds (**Harborne et *al*., 1980).**

Carbone skeleton	Class	Example
C6	Simple phenols	catechol
C6-C3	Hydroxybenzoic acids	P-hydroxybenzoic
C6-C3	Hydroxycinnamic acids	Coffee Acids, féruliquescopolétine, esculetin, coumarins
C6-C4	Naphtoquinones	juglone
C6-C2-C6	Stilbene	esveratrol
C6-C3-C6	Flavonoids, Flavonols, Anthocyanins, Flavanols, Flavanones, isoflavonoids	Kaempferol, Quercetin, Pelargonidine, Cyanidine Catechin, Epicatechin Naringenin, Deidzime
(C6-C3)2	Lignans	Pénorésinol
(C6-C3) n		___
(C15) n	Tannins	___

III.2. Enrichment of vegetable oils

III.2.1.Extraction solid liquid

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 (**Xiuzhen et *al*., 2007).**

III.2.2.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 **(Xiuzhen et *al*., 2007).**

III.2.3.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 **(Xiuzhen et *al*., 2007).**

III.2.4. ultrasound-assisted enrichment

In recent years, different studies have used olive 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).** Ultrasound is a mechanical wave that is able to move in an elastic medium at a frequency higher than the maximum audible limit of the human ear (16 kHz) **(Achat et *al*., 2012)**

- Table IV present example of some works of enrichment of edible oils with antioxidant

Table IV: Different types and example of enrichment of edible oil.

Vegetable matrix	Oil	Compound	Condition of extraction (results)	Reference
olive leaf , lemon balm	corn oil	Polyphenols	- Solid–liquid extraction method: 4000–10 000 rpm and 30–90 min -Total phenolic content increased 9.5 and 2.5 times over the pure corn oil. - Antioxidant activities of enriched oil extracts 14 and 6 times higher than that of untreated oil	**(Ahin et al; 2016)**
Olive leaf	Refined olive refined soybean oil	Antioxidant (oleuropein)	- Better oxidative stability, Rancimat method, the fresh oil samples with 4.25 h for refined olive oil and 3.80 h for refined soybean oil	**(Zribi, 2013)**
Olive leaves	Olive oil	Phenolic compound (oleuropein)	- Ultrasonic enrichment (60w,16°C,45min) →414,3 ±3,2mg O/kg of PT oil (be an I ncrease of 132mg compared to oil witness) et 111,0 ± 2,2mg 0 /kg of oleuropein oil with a %DPPH of 86,2 ± 0,2%.	**(Achat et al; 2012)**
Green tea White tea	Virgin olive oil	Phenolic Compounds	Enrichment (liquid-liquid), microware (2g / 250ml distilled water +1mg/ml of olive oil) -TP oil (524.6mg CAE/kg)	**(Malheiro; 2012)**

			-TP enriched white tea oil(580.55 mg CAE/kg) -TP enriched green tea oil(636.5 mg CAE/kg) -DPPH% enriched green tea oil is of 87.7%and 58.4 exposed by microware for 1min to 5min respectively	
Tyms and pomace	Olive oil	Phenolic compounds	Liquid-liquid extraction 2,5g of extract /100g of oil -TP of virgin olive oil(74mg/kg of oil) -TP of enriched oil pomace (365kg/ kg of oil) -TP of enriched thyme oil (268mg/100g of oil) Antioxidant power of dry thyme is from 131.10^3 to 139, 4.10^3 µmol TE/100g calculated by rancidity test.	**(Rubio; 2012)**
Olive leaves (kalamori variety)	Table olive	Polyphenol Oleuropein and hydroxyl-tyrosol)	The antioxidant activity of extracts was determined using the Rancimat method and their content in oleuropein and hydroxytyrosol was determined by HPLC	**(Lalas, 2011)**
sage and rosmary leaves	Olive oil	Carnosic acid	solid-liquid extraction (0.1,0.01mg E/100g of olive oil),60°C et 180°C -tyrosol :11.4 mg/kg Hydroxyterosol:10.8 mg/kg DPPH %:81.6	**(Zunin; 2010)**
			-Conventional aromatisation (150g of leaves /1L of oil under agitation for 12 hours at room temperature)→ 1,66mg/L of eugenol oil	

Basil or basilic leaves	Olive oil	Essentiels oils (Linalool and Eugenol)	-aromatisation with ultrasound method (1W, 25KHz): 1/150g of powder in 1L of olive oil for 15min→3,68mg /L , 1,34mg/L of linanool and eugenol oil respectively 2/50g of powder in 1L of olive oil for 15min →1,94mg/L of linanool oil and 0,79mg/L of eugenol oil	**(Veillet; 2010)**
rosemary, lavender, sage, menthe, basil, lemon and thyme	Olive oil	chlorophyle carotene polyphenol	direct enrichment (solid-liquid) 5g/100ml of olive oil for 15days Chlorophyle (2.14-3.89mg/kg of oil) Carotene (2.85-6.81 mg/kg of oil) Polyphenol (50-1000 mg/kg of oil)	**(Ayadi; 2009)**
Olive leaves	Refined oil	Total polyphenols	Enrichment after extraction using 120mg of total polyphenols extract(PT)/kg of oil and 240mg of PT/kg of oil→ passage or transition of 8,2mg Caffeic acid equivalent(CAE)/100mg of oil at 14,7±0,7 mg CAE/100mg of oil and at 20,2± 0,9 mg CAE/100mg of oil respectively	**(Chiou; 2009)**
Tomato	Olive oil	carotenoid	Enrichment solid-liquid) 0,5mg (E1) and 1,0 mg (E2) of lycopene in 100mg of oil) Liquid-liquid : 2g of olive oil(E3) in to methanol/water -TP (mg/kg of oil): E1(410),E2(450),E3(500) -α-tocopherol content (mg/kg of oil): E1(116),E2(119),E3(119) Lycopene concentration (mg/100kg of oil): E1(0,37±0,01); E2 and E3(0,56±0,02)	**(Montesano ; 2006)**

Experimental Part

Material and Methods

I. Materials and methods

I.1. Work plan

The direct enrichment of oils (olive oil and refined oil), with plant materials, was performed using an optimization method experiment designs **(Fig.2).** This work has been done in block 11.

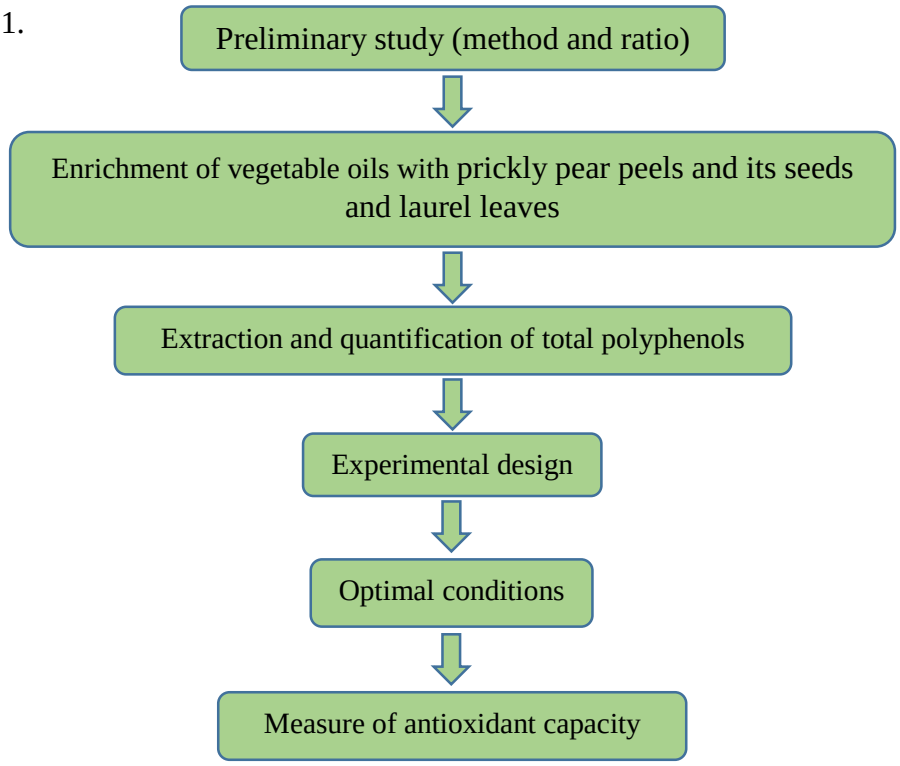


Figure 2: General work plan

I.2. Samples

I.2.1. Plant material

- The laurel leaves used were bought from market (Bejaia). The sample was cleaned with distilled water, dried in the drying oven at 40 °C until constant weight. The dried laurel leaves was ground using a grinder (IKA A11 BASIC, Germany) and sieved to <1 mm particle size.

- The dried peels and seeds of prickly pears (*Opuntia ficus indica* L) were obtained from laboratory 3BS (Bejaia).

- **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 an oven (BINDER) to dryness at 103 ± 2 ° C, until constant weight. The moisture content (MC) was calculated by the following formula **(Doymaz et al., 2004).**

$$MC\ \% = \frac{Wi - W0}{Wi} \times 100$$

- Where W_0 correspond to the loss in weight (g) on drying and W_i correspond to the initial weight of sample (g).

I.2.2. Vegetable oils

- The olive oil used in this work, was obtained near Fénaia Il-Mathen (Elkseur) in Bejaia, for Chemelal variety and during the harvest period of the year 2017.
- The refined oil (sunflower and soya) was supplied from market (Bejaia) produced by CEVITAL (elio).

I.3. Extraction procedures

I.3.1. Preliminary study

A preliminary study was performed in order:

- To select the better method for enrichment, using conventional maceration (CM) and ultrasound assisted maceration (UAM).
- To determine optimal solid–liquid ratio for the rest of investigation. Amount of plant material subjected to enrichment of oils varied from 5 to 20 g for 100 mL of oil under stirring during 1h to3h. Experiments were followed using Folin–Ciocalteu's reagent after extraction of total phenolic compounds. Results were reported as mg of gallic acid equivalent per kg of oil (mg GAE/ Kg oil) (**Fig.3**).

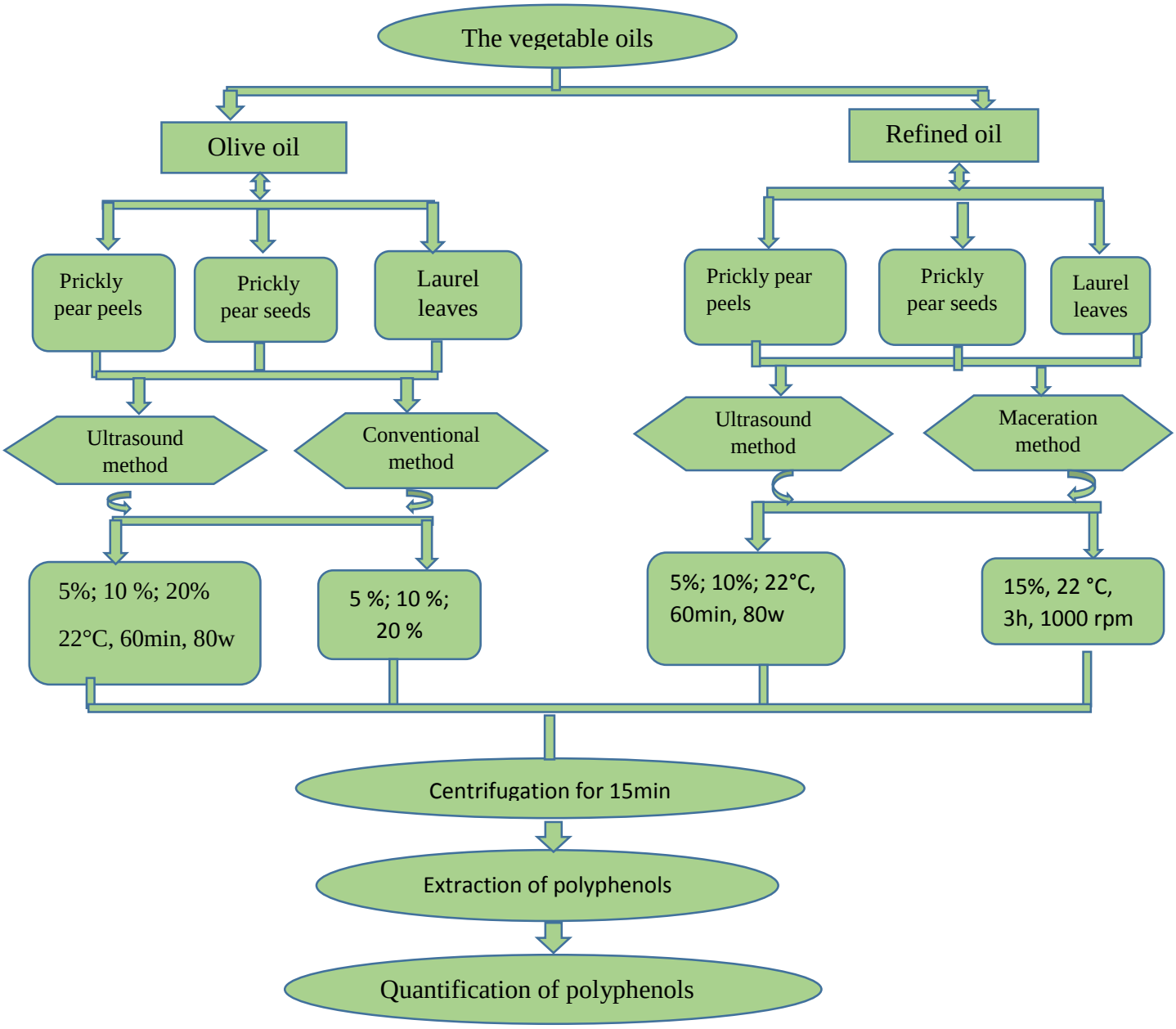


Figure 3: Steps of the preliminary study.

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 80 w. Different amount of dried and milled plant materials were added to different volume of oils, as solvent into the ultrasonic device during different times.

b- Conventional maceration (CM)

Different amount of dried and milled plant materials were added to oils, under stirring, during 3 h for preliminary study (**Penalvo et *al*., 2016).** Conventional, made for comparison,

was carried out in the exactly same conditions without ultrasound, under the optimal conditions given by experiments plan.

I.3.2. Extraction of polyphenols from oils

The extraction of the phenolic compounds (**Fig.4**) is carried out following the procedure described by **Tsimidou et al., (1992**).

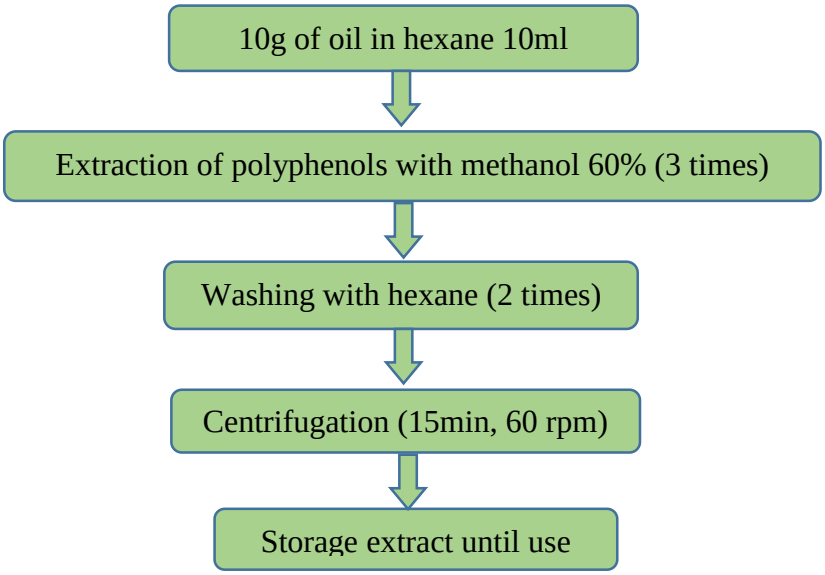


Figure 4: Extraction of polyphenols from oils (**Tsimidou et *al.*, 1992**)

I.4. Determination of polyphenols from oils

I.4.1. Total phenolic content

Total phenolic content (TPC) of oil extracts (**Fig.5**) was determined by the method of **Negi et *al., (*2003**). Oxidations of phenolic compounds with this reagent include reaction with the mixture of $H_3PW_{12}O_{40}$ and $H_3PMo_{12}O_{40}$ acids in the alkaline medium. At this reaction a mix of blue oxides is formed **(Ribbreau-gayon et *al.*, 1968).**

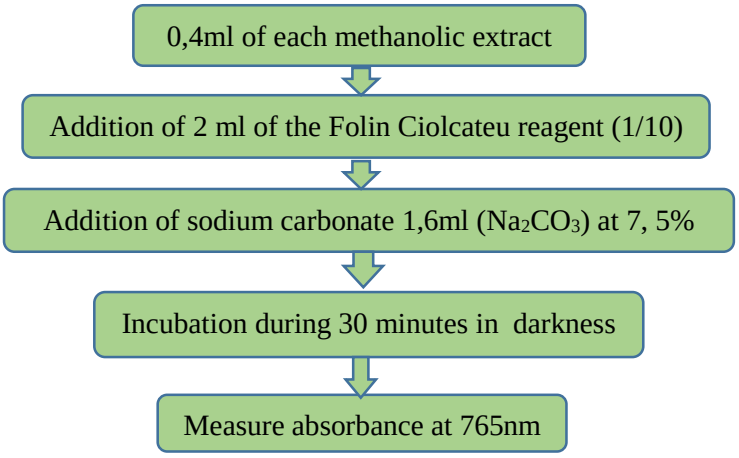


Figure 5: Determination of total phenolic content of oils (**Negi et *al.*, 2003**).

I.4.2 Flavonoïds content

The total flavonoid content (TFC) was determined (**Fig.6**), according to the mostly applied colorimetry method based on the formation of aluminium-flavonoid complexes and following the procedure of **Ghafar et *al*., (2010**)

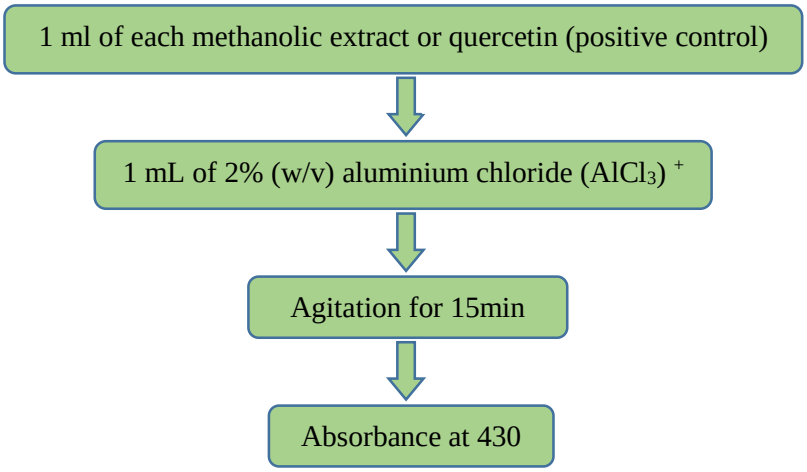


Figure 6: Determination of TFC in oils **(Ghafar et *al*., 2010).**

I.4.3. Betalains content

Betacyanins and betaxanthins content was reported as mg equivalent betanin/L and mg equivalent indicaxanthin/L, respectively. Betacyanins were detected at 538 nm and betaxanthins at 480 nm **(Martínez et *al*., 2011**).

$$\text{Betacyanins or betaxanthins content [mg/L]} = [(A * DF * MW * 100/ \varepsilon * 1)]$$

Where: A = absorbance at 535 or 480 nm; DF = dilution factor;

MW = molecular weight; Ɛ = extinction coefficient;

l = width of the spectrophotometer cell (1 cm);

The Ɛ of betacyanin is 60,000 L/ (mol.cm) and MW = 550 g/mol. For betaxanthins the Ɛ is 48,000 L/ (mol.cm) and MW = 308 g/mol.

- Total betalains (betacyanins + betaxanthins) were expressed as mg/100g of dry weight (DW).

I.5. Antioxidant activities

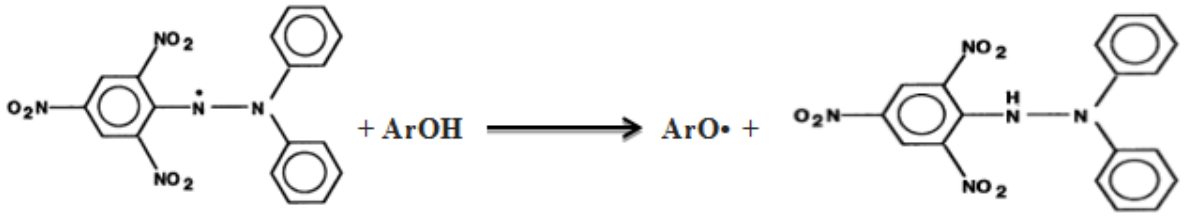
I.5.1. DPPH° assay

The antioxidant activity of oils, was evaluated according to the method of the free radical-scavenging activity (RSA) (**Achat et *al.*, 2012**). DPPH° (1,1-diphenyl-2-picryl-hydrazyl) is a stable highly colored free radical that can abstract labile hydrogen atoms from phenolic antioxidant (ArOH) with concomitant formation of a colorless hydrazine (DPPH-H), according to equation 1 and figure 10 **(Molyneux 2004)**. The RSA of an extract **(Fig.12)**, can be expressed as the percentage of DPPH reduced by a given amount of extract. The total RSA of each extract was expressed as the percentage of DPPH reduced and was calculated by the following equation:

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

A_0, absorbance of DPPH solution without any antioxidant; A, absorbance of DPPH solution after reaction with the extract.

$$\text{DPPH}^{\bullet} + \text{ArOH} \xrightarrow{k} \text{DPPH-H} + \text{ArO}\bullet \qquad \text{[Eq.1].}$$



Diphenylpicrylhydrazyl (free radical)

Diphenylpicrylhydrazine (nonradical)

Figure 7: DPPH° radical reduction **(Molyneux 2004)**.

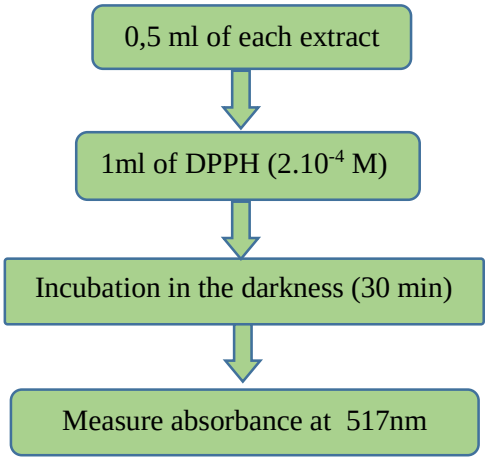


Figure 8: Steps of DPPH° test (**Goupy et *al.*, 2003; Achat et *al.*, 2012**).

I.5.2. Reducing power

The reducing power of different extracts **(Fig.9)** were measured according the method of **(Zhou et *al.*, 2004).** A higher absorbance indicates a higher reducing power.

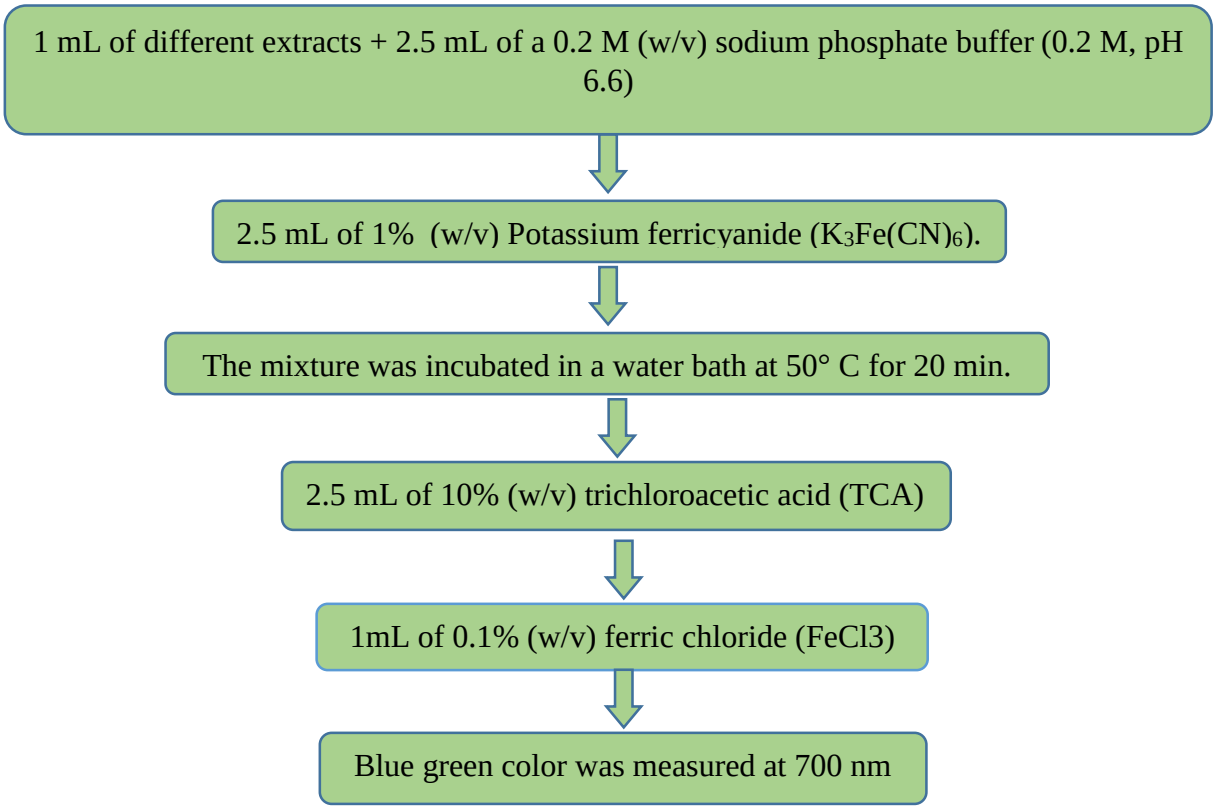


Figure 9: Steps of the reducing power assay (**Zhou et *al.*, 2004).**

I.6. 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).**

I.6.1. 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 three-level Box-Behnken plan to evaluate the combined effect of three independent variables: temperature, time, ratio, which are designated X1, X2 and X3 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.

Configuration	Temperature (°C)	Temps (min)	Ratio %
000	32,5	37,5	12,5
−−0	20	15	12,5
++0	45	60	12,5
+−0	45	15	12,5
+0+	45	37,5	20
0+−	32,5	60	5
−0+	20	37,5	20
000	32,5	37,5	12,5

0++	32,5	60	20
0−−	32,5	15	5
+0−	45	37,5	5
000	32,5	37,5	12,5
0−+	32,5	15	20
−0−	20	37,5	5
−+0	20	60	12,5

- Thus 15 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 below.

$y = a0 + a1\ X1 + a2X2 + a3X3 + a4X4 + a12X1X2 + a23X2X3 + a34X3X4 + a13X1X3 + a14X1X4 + a24X2X4 + a1X1^2 + a2X2^2 + a3X3^2 + a4X4^2 + E$

With: y: measured response, X1, X2, X3 and X4: are the factors studied, E: error a0, a1, a2, a3, a4, a12, a23, a34, a13, a14 and a 24: are the regression coefficients.

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Results and Discussions

II. Results and discussion

II.1. Choice of matrix

a- Peels and seeds of prickly pear

Several works have been studied the prickly pear, seeds, fruits and peels. Peels constitute a source of bioactive compounds, namely phenolic acids, flavonoids and betalains (**Arrizon, Calderon & Sandoval., 2006, Kuti., 2004)**. Phenolic compounds represent very interesting molecules due to their ability to reduce or prevent oxidation (**Achat et *al*., 2016).** The prickly pear seeds are characterized by their hardness due to the presence of fibers. Cactus seeds can be used to prepare cactus oil, rich in essential fatty acids and liposoluble antioxidants. (**El-Mostafa et *al.*, 2014).**

b. Laurel leaves

This aromatic plant contain essential oil rich and polyphenols; very important antioxidant molecules**.** It is already known that maceration of these plants can lead to enrichment of oils that increased its nutritional value **(Jović et *al*., 2018).**

c- Vegetable oils

The choice of olive oil (chemlal variety), comes from its low content of antioxidant that makes it sensitive to oxidation;

- Refined oils are susceptible to oxidation because they contains large amounts of unsaturated fatty acids, particularly polyunsaturated fatty acids, such as linoleic acid (18:2 w-6). Lipid oxidation produces rancid odors, unpleasant flavors, and discoloration. It also decreases the nutritional quality and safety of foods due to secondary oxidation products that have harmful effects on human health **(Mezza et *al*., *2017)*.**

II.2. Choice of methods: extraction and quantification of polyphenols

Phenolic compounds play a very important role in the characterization and nutritional value of oils **(Brenes et *al*., 2002)**. These are the main compounds responsible for the stability of olive oils during storage and heating **(Dimitros et *al*., 2006).**

- In order to extract the polyphenols contained in the studied oils, a liquid - liquid extraction was carried out with two solvents, hexane and methanol:

Methanol has shown better yields as extraction solvents for polyphenols compared to the other solvents mentioned in the literature (acetone, ethanol, etc.). This solvent is less polar than water and very effective for the release of polyphenols (**Johns et *al*., 1999, Sacan et *al*., 2010).** For this reason we have used it in this study, compared to other organic solvents. In addition, when added for extraction, two phases are formed **(Appendix 1**), an upper phase, hexanic extract containing lipid fractions and a pellet phase, methanolic extract, containing water-soluble compounds with polyphenols.

- The quantification method of total polyphenols by the FC reagent is a rapid and economic analysis that is widely used to estimate the phenolic content of vegetable oils. When the FC reagent is added to the methanolic extracts of oils, a yellowish color is formed **(Appendix1**). However the addition of sodium carbonate, alkalinizes the medium that gives a blue color **(Appendix1),** which could justify the presence of polyphenols content in extracts.

II.3. Preliminary study

The effects of the variable of solid–liquid ratio and the method of enrichment of oils (CM and UAM), on the rate of TPC, betalains and DPPH°, were evaluated using cactus peels and seeds and laurel leaves.

II.3.1. Prickly pears

II.3.1.1. Peels

a. Total phenolic content (TPC)

Figure 10 depicted the amount of TPC of cactus peels, using two enrichment methods (CM and UAM).

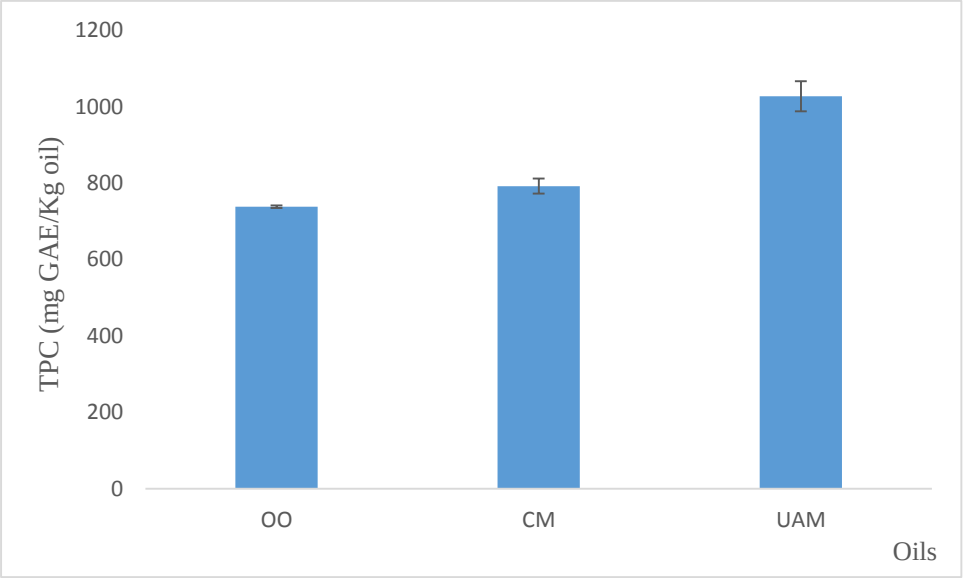


Figure 10: The TPC of the olive oils extracts using CM and ultrasound assisted UAM.

Polyphenolic content presented differences according to enrichment method used. The result obtained by UAM was the richest in phenolics (1027.16 ± 13.42 mg GAE/kg oil) for dried cactus peels. However, the lowest one was attributed to OO and CM (738 ± 5.6; 791.97 ± 10.6 mg GAE/ Kg oil) respectively.

Some studies on the effect of UAM showed that the cavitation phenomena is responsible for modification on the plant material inducing disruption of the cells, due to the burst of the cavitation bubble on the surface matrix: Ultrasound propagates mechanic waves through an elastic medium. The sound wave dislodges the molecules of the medium from their original location, thereby creating areas of compression and rarefaction corresponding to the compression and rarefaction cycles of the waves. (**Veillet et al., 2010, Achat et al., 2012; Penalvo et al., 2016).**

- Results of ratio effect on TPC revealed a proportionality between ratio and polyphénols contents **(Fig.11):** 1027.16 mg GAE/kg oil and 1241.39 mg GAE/kg oil, corresponding to 5% and 10% ratios**.** Solid-liquid ratio present an important factor in the enrichment of oils.

Figure 11: Effect of ratio on TPC rate by UAM

b- Betalains content

Betalains are the characteristic pigments of the prickly pear. Spectrophotometric quantification of these substances has been reported **(Fig.12):** for the two extraction methods. Results indicated a differences between the enrichment methods (UAM and CM). UAM exhibited the highest betalains contents: 35.08 ± 0.05 mg/100 g DM.Whereas the amount obtained with OO and CM was the lowest (23 ± 0.96 mg/100 g DM and 31.31 ± 2.12 mg/100 g DM) respectively.

Betalains such as betacyanins and betaxanthins occur in a number of natural sources, however, prickly pears, beets, and the fruit from vine cactus are the only foods containing this class of compounds. Consistent with the results of others, we found that as betacyanins and indicaxantin are the main betalain pigments of the prickly pear **(Butera et *al.*, 2002).**

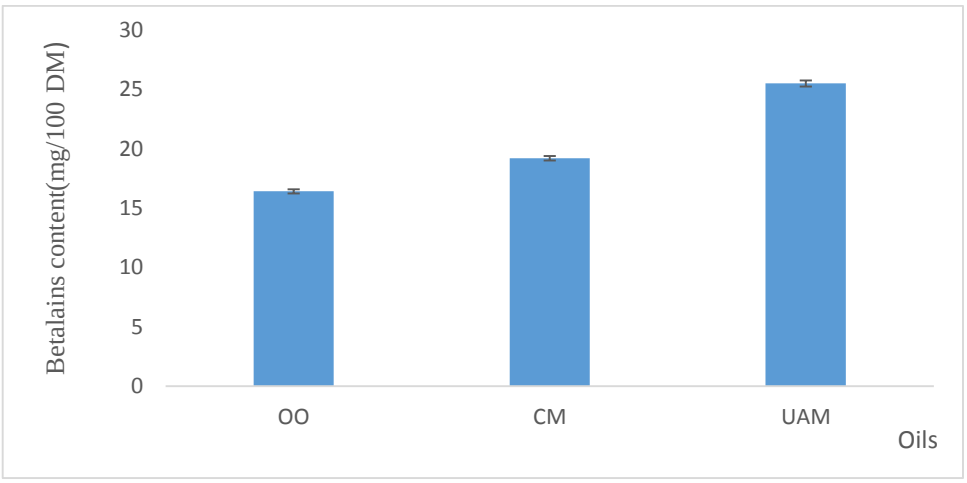


Figure 12: Betalains content in peels of prickly pear

c. DPPH° assay

The DPPH radical is usually used as substrate to evaluate the antioxidative action of antioxidants by determining the free radical-scavenging ability of various samples **(Achat et *al*., 2012).** This purple chromogic, allows a fast analysis of the antioxidant activity of a great number of samples and gives reproducible results **(Gulçin et *al*., 2010).**

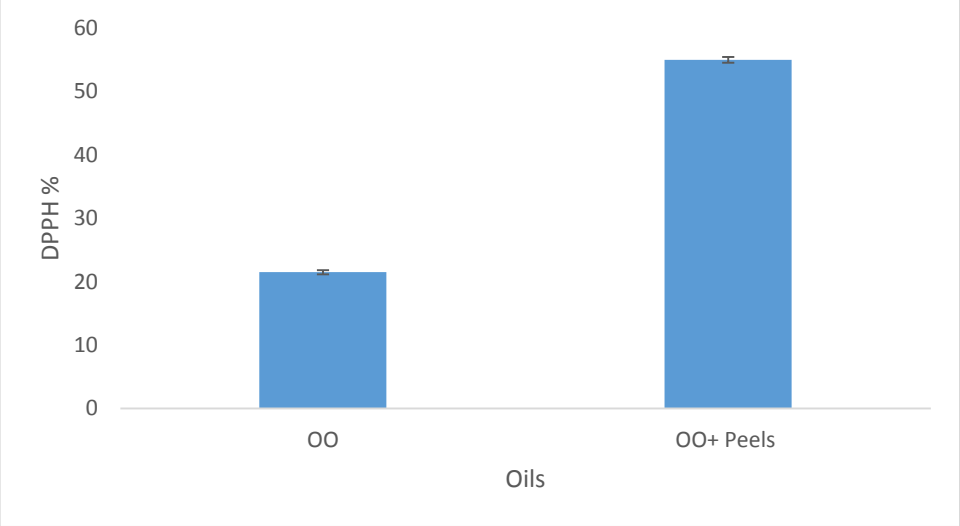


Figure 13: Antiradical capacity of the analyzed olive oils; OO and OO with peels using UAM

The best antioxidant activities were shown by enriched OO (UAM): 55.6 ± 1.13 %, compared to OO (21.5 ± 1.90 %). These results are in accordance with the amount of bioactive components (TPC, and betalains) quantified in cactus peels extracts. The enrichment yield increases the scavenging activity for the peel of prickly pear fruit. UAM produces 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 **(Ramli, Ismail et *al*., 2014).**

II.3.1.2. Seeds

The data of effect of extraction methods and ratio, on TPC and the radical scavenging activity of olive oil, with cactus seeds, were shown in figure 14, 15 and 16 respectively.

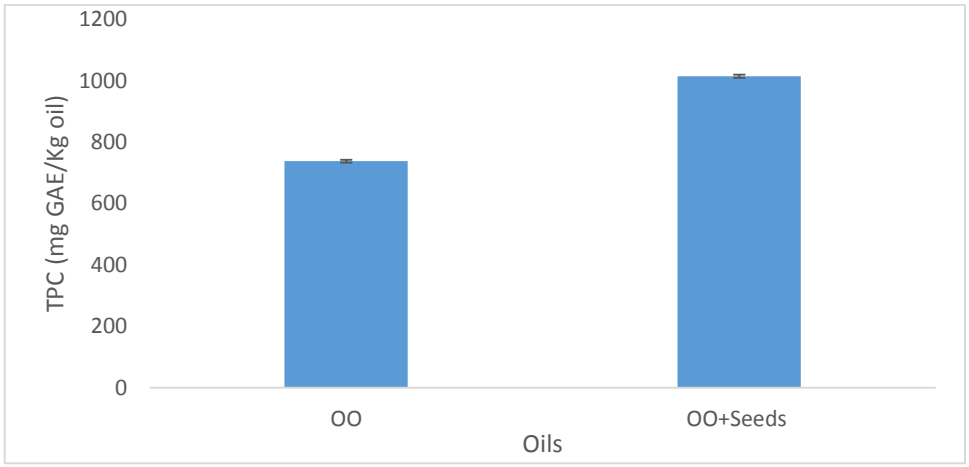


Figure 14: TPC of the olive oils extracts

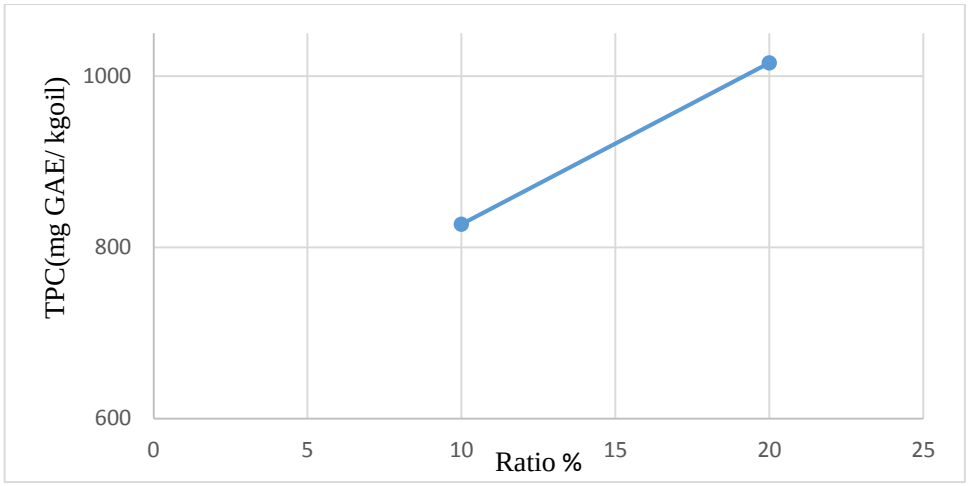


Figure 15: Effect of ratio on TPC value

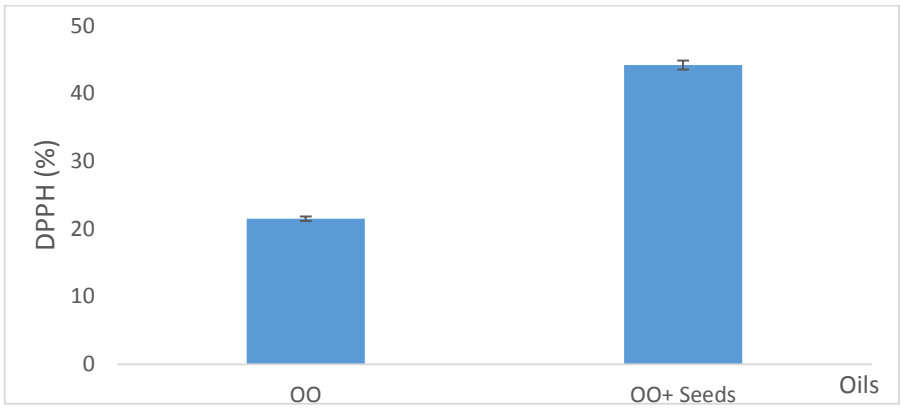


Figure 16: Antioxidant activity (DPPH°) of olive oil extracts

Figures, above, showed the same tendencies of results obtained in the enrichment of OO with peels.

II.3.2. Laurel leaves

a. Moisture content

The drying efficiency was evaluated in terms of water loss, the value obtained is about **15%**; the fresh plant material contains a 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)**. In addition, moisture also help the development of microorganisms and molds that cause massive and rapid degradations of vegetable matrix during storage **(Johns., 1999).**

In order to limit these reactions and ovoid oxidation or degradation of polyphenols, drying at 40 ° C was applied. This temperature allowed the dryness of laurel leaves without a harmful modifications during conservation and use.

b. TPC and DPPH° test

Figures 17, 18and 19 show that the addition of laurel leaves in OO, under sonication, yielded higher values of TPC (1090.3 ± 10.07 mg GAE/Kg oil), antiradical capacity (51.46 ±2.1 %) in comparison with the OO: 738.3 ± 4.7 mg GAE/Kg oil and 21.5 ±2.1 % respectively. These results confirmed, that laurel leaves contain antioxidants compounds that are transferred to OO.

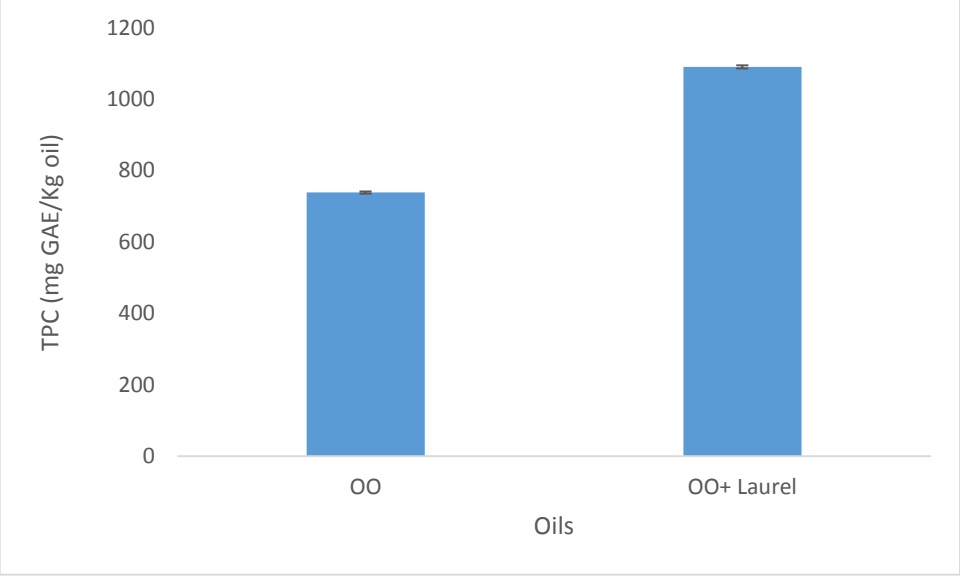


Figure 17: TPC in olive oil extracts enriched with laurel leaves

Free-radical scavenging activity is mediated by an electron donor molecule (antioxidant). Phenols are H-donor molecules. Oregano, rosemary, and laurel essential oils with

phenolic components have shown remarkable antioxidant activity because these compounds have a phenolic base. **(Mezza et *al.*, *2017).***

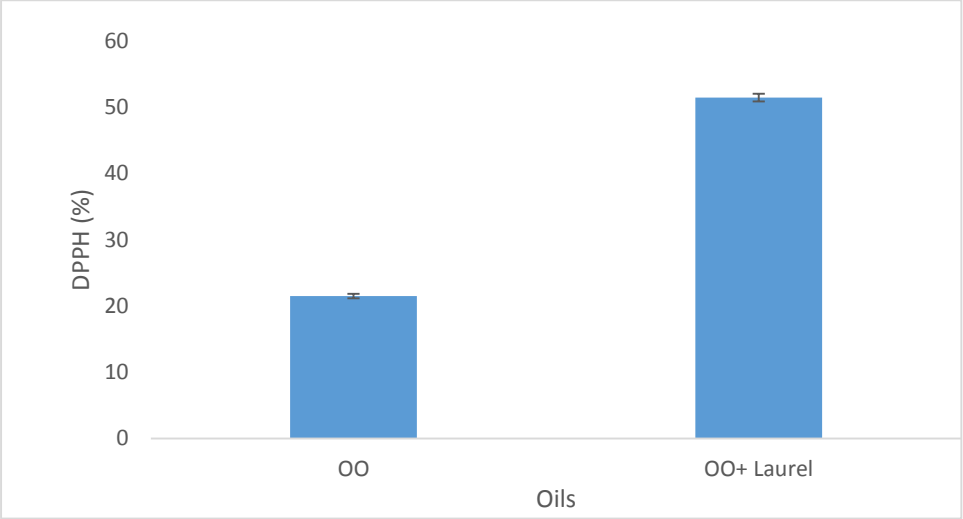


Figure 18: Reduction of DPPH in olive oil extracts

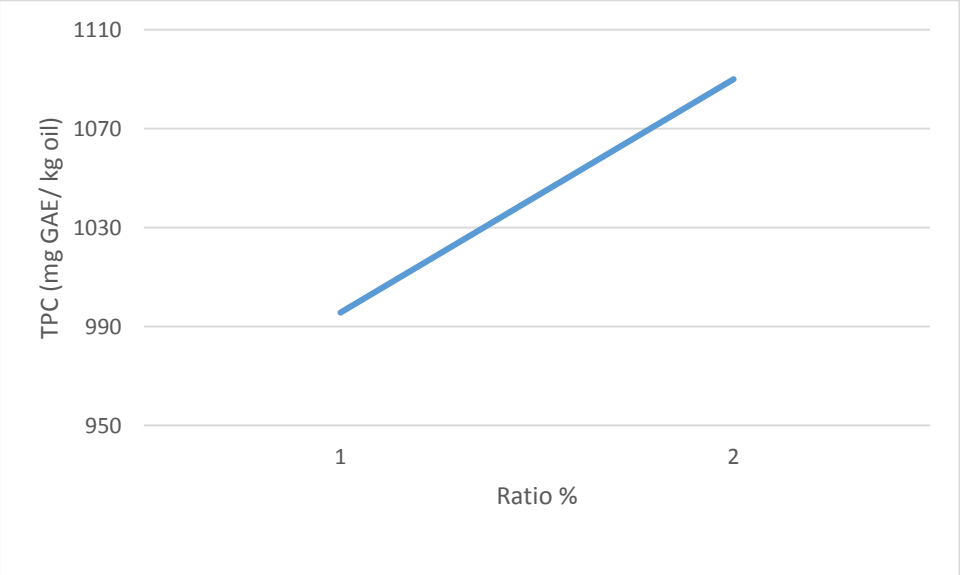


Figure 19: Effect of the ratio on TPC rate

Solid-liquid ratio, also using laurel leaves play an important role in the enrichment of oils.

II.4. Refined oil

Results of addition of different plant material (cactus peels, seeds and laurel leaves) using the ultrasound method, were presented in figure 20.

The great value of TPC was attributed to the laurel leaves (313.43 ± 1.64mg GAE / kg oil). The lowest one was obtained by RO (89.88 ± 4.69 mg GAE/kg oil). This can be related to their low content of phenolic compounds. Indeed, according to **Papadopoulos et Boskou., (1991**), the refining of an oil is at the origin of the removal of any trace of polar compounds such as polyphenols. Thus, the minute rate obtained in polyphenols of sunflower - soy oil can be explained by the existence of other compounds that absorb at the same wavelength as the phenolic compounds **(Chimi et *al*., 2001).**

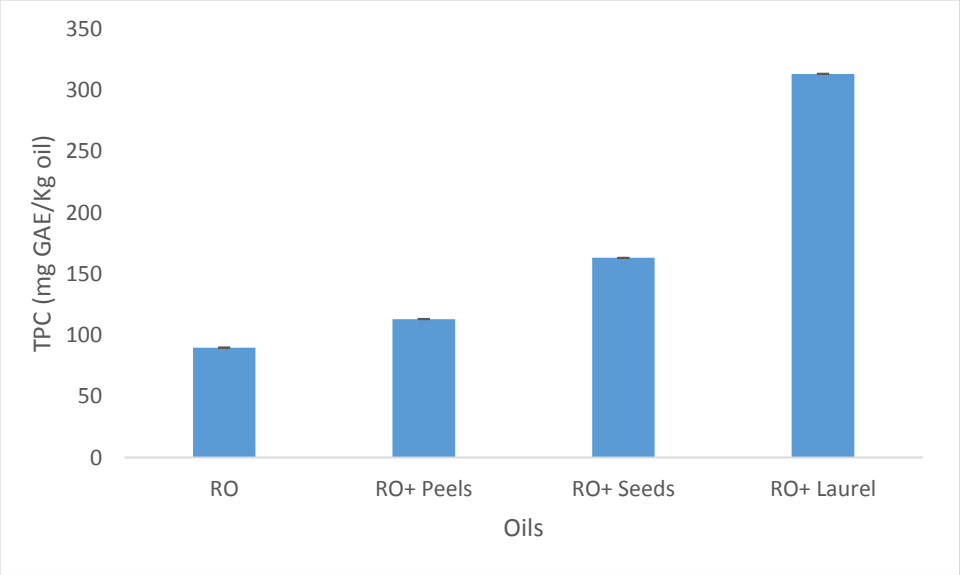


Figure 20: TPC in refined oils extract: before and after addition cactus peels, seeds and laurel leaves.

It is noticed that the increase on **TPC** oil extracts, can directly related to their enrichment by polyphenols of the used plant materials.

II.5. Comparison olive oil- refined oil

According to the results depicted in the figures 21 and 22, the highest concentration of TPC, and the greatest antioxidant activity were obtained for olive oil before and after addition of selected plant materials. Thus it applied for the rest of investigation: OO using cactus peels and laurel leaves under sonication.

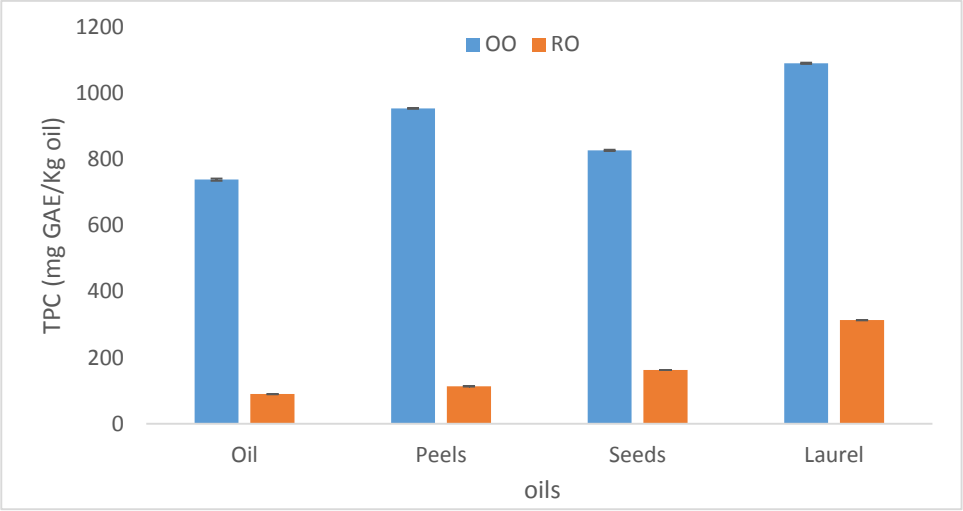


Figure 21: TPC rate in both olive oil and sunflower-soy oil

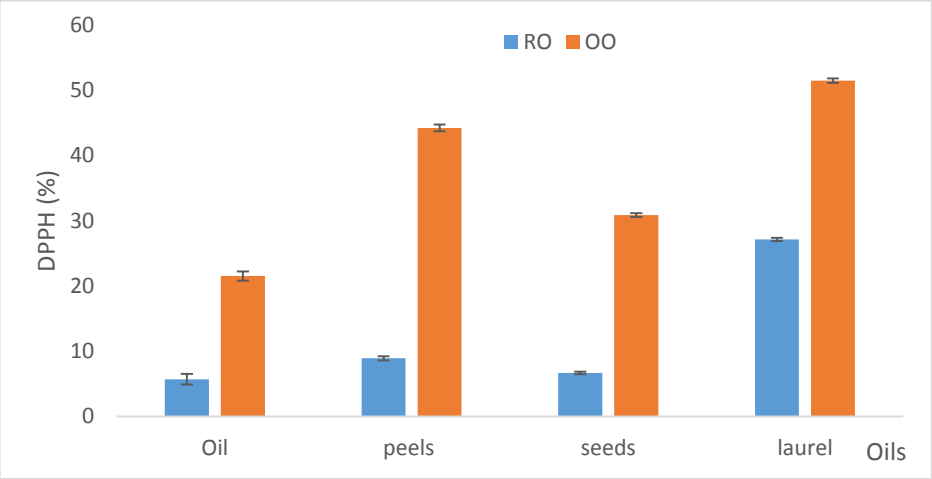


Figure 22: DPPH inhibition for olive oil and sunflower-soy oil extracts

II.6. Optimization of extraction 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 **(Wang et *al*., 2009).**

II.6.1. Experimental design of OO-cactus peels

Fifteen experimental points run randomly according to the UAM (OO-peels) experiment planning, the triple coded values of independent variables and responses obtained in the multivariate study for each experiment are shown in Table VII.

Table VI: Fully coded box behnken design (BBD) and responses obtained from OO-catus peels.

Run	Temperature (°C)	Time (min)	Ratio (%)	Experimental TPC (mg GAE/kg)	Predicted TPC (mg GAE/kg)
000	32,5	37,5	12,5	1055,11	1057,05
−−0	20	15	12,5	970,47	995,42
++0	45	60	12,5	885,12	860,17
+−0	45	15	12,5	843,89	822,47
+0+	45	37,5	20	824,57	863,15
0+−	32,5	60	5	899,74	916,89
−0+	20	37,5	20	1163,26	1155,46
000	32,5	37,5	12,5	1020,18	1057,05
0++	32,5	60	20	950,32	936,69
0−−	32,5	15	5	901,79	915,41
+0−	45	37,5	5	991,14	998,93
000	32,5	37,5	12,5	1095,86	1057,05
0−+	32,5	15	20	911,25	894,09
−0−	20	37,5	5	1059,76	1021,19
−+0	20	60	12,5	1015,37	1001,79

a- Analysis of the variance and validation of the model

In the experimental design, 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 adjustment models obtained for the different responses are summarized in the figure below:

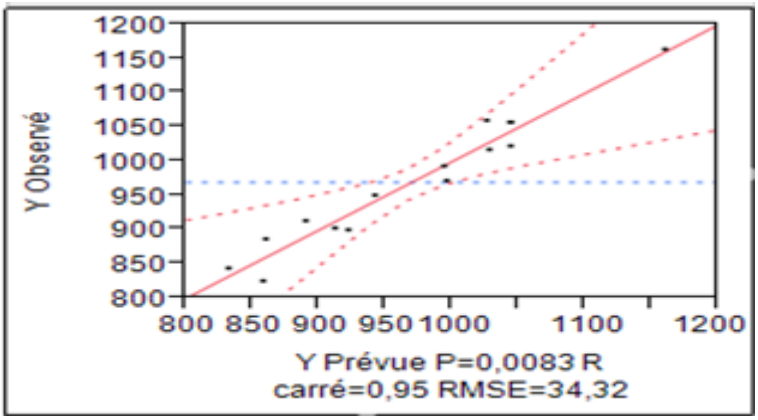


Figure 23: Comparison between predicted values of TPC and experimental values.

As can be seen, a good quality of fit is obtained for TPC 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 86%)** and the Lack of fit values are greater than 0.05 **(Tab.VIII**), 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 between 0.0001 to <0.0022.

Table VII: ANOVA TPC obtained in the BBD (OO-peels)

Source	Sum of squares	*Df*	Mean squares	F-ratio	*P*-value
Linear effect					
X1 : Temperature	55137,036	1		34,9143	**0,0020**
X2: Time	1895,748	1		1,2004	0,3232
X3: Ratio	1,146	1		0,0007	0,9795
Interaction effect					
X2X3	422,757	1		0,2677	0,6269
X1X2	3,370	1		0,0021	0,9649
X1X3	18232,640	1		11,5454	**0,0193**
Quadratic effect					

X1X1	1094,903	1		0,6928	0,4431
X2X2	45594,261	1		28,8716	**0,0030**
X3X3	3356,757	1		2,1256	0,2047
Lack-of-fit	5026,6030	3	1675,53	1,1678	0,4921
erreur	2869,4431	2	1434,72		
total	131105,94	14			

b. Response Surface Analysis

Response surface analysis as expected and according to the response surfaces, the extraction efficiency in terms of TPC increases by increasing temperature and time. Thus, the finally values selected correspond to the maximal values chosen to define the experimental domain. After, the observations made during the study of the response surfaces **(Fig.24).** Thus, the optimization of the temperature and the ratio is at the maximum of these two parameters at 32 ° C. and 37.5 min for TPC.

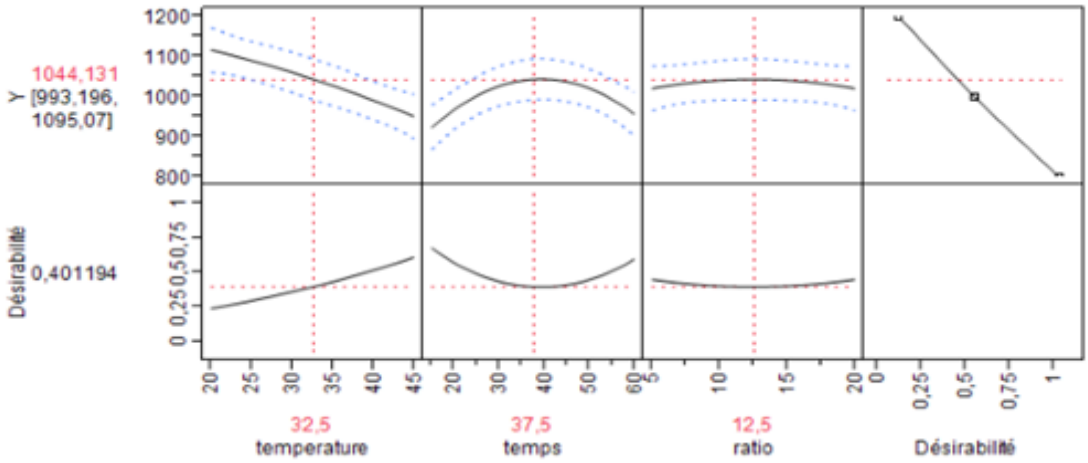
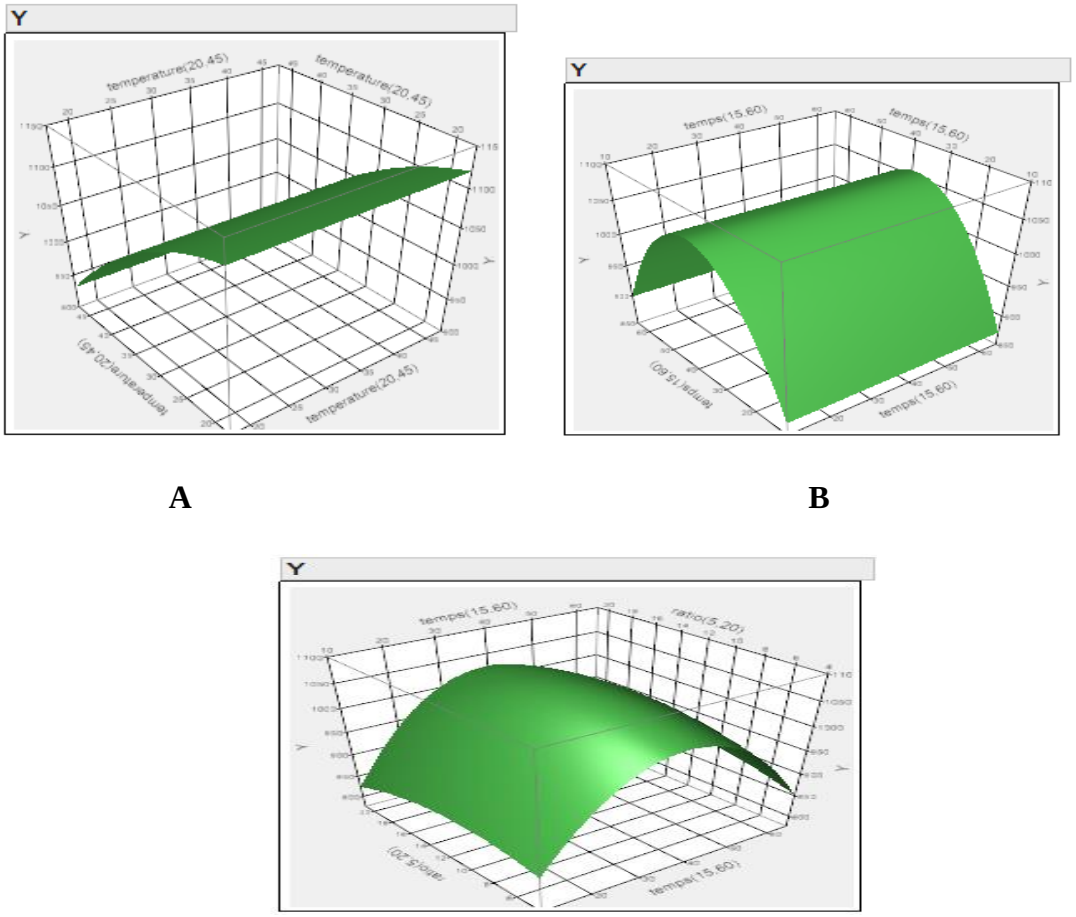


Figure 24: Graphical interpretation of results by JMP: General Effect of Factors



A **B**

C

Figure 25: Response surfaces of the effect of different factors:

A: temperature influence

B: influence of time

C: ratio influence and time

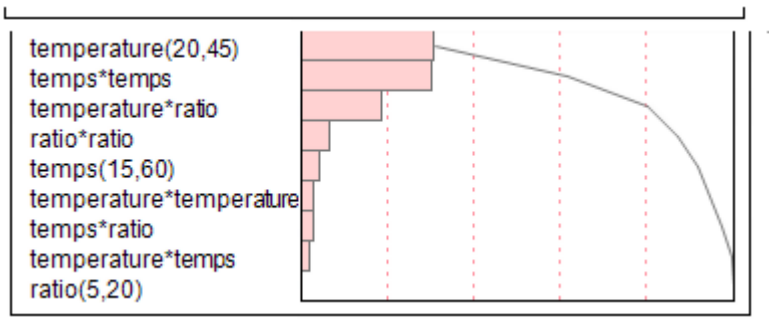


Figure 26: Standardized Pareto diagram for TPC concentration.

According to the results of the Pareto Diagram, that determines which parameters have a significant influence on the final concentration of TPC. It has been found that the linear effects of temperature are significant (**Achat., 2013**), followed by the quadratic effects of time (X12), and the interaction effect (X1X3) (temperature, ratio) have little significance on the polyphenol contents.

c. Verification of predictive model

The optimal conditions retained in this study are 32 C°; 37, 5 min and 12, 5% for temperature, time and ratio respectively. The experimental results of TPC was very close to the predict one. This implied that there was a very high fit degree between the values observed in experiment and the value predicted from the regression model. Hence the response surface modeling could be applied effectively to predict enrichment of OO with antioxidants substances from cactus peels.

The predicted model was described by the following second-order polynomial equations:

$$\mathbf{TPC = 1057,05 – 83,02*T - 67,51*T*R – 111,1236*t\text{^}2.}$$

With :

T : Temperature ;

t: Time ;

R : Ratio.

II.6.2. Comparison of UAM and CM (OO-peels)

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 **(Li et al., 2012).**

A comparison was carried out between UAM and conventional maceration method without application of ultrasound (CM), for the enrichment of OO with polyphenols of cactus peels (**Tab.VIII**), using optimal conditions. It is observed that the highest content of TPC the best antioxidant activities (Reducing power and DPPH) were assigned to UAM. Sonication

improved the extraction efficiency by and favoring the solubilization of the targeted compounds. The marked increase in the very local temperature enhances the solubility of the analytes in the solvent and eases their diffusion from the sample matrix to the outer region.

Table VIII: Comparison of the OO extracts before and after addition dried peels.

	Experimental value OO	Experimental values: EAM	CM
TPC (mg GAE/kg il)	738,47 ± 3,29	1057,43 ± 19,75	794,29 ±9,87
Betalains (mg/100g DM)	1,19 ± 00,2	261 ± 0,004	133 ± 0,021
Flavonoid (mg QE/ kg oil)	14,398±0,667	34,06 ± 4,22	21,63 ± 0,44
Reducing,power	0,069 ± 0,014	0,131 ± 0,002	0,115± 0,002
DPPH (%)	21,5 ± 0,02	88.09 ± 0,02	68,25 ± 5,05

Moreover, the implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate the cells (Fig.27) **(Achat et al., 2012).**

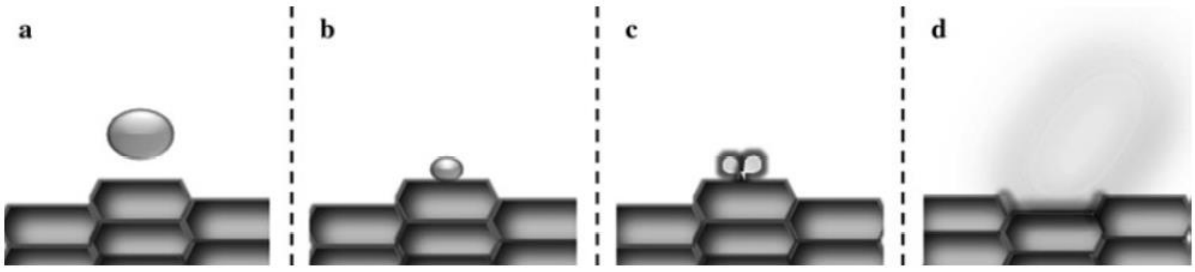


Figure 27: Schematic representation of the rarefaction **(a)** and compression cycle **(b)** of a cavitating bubble that eventually collapses near the solution/biological tissue interface generating shock waves and microjets **(c)** that can disrupt the cell wall releasing the intracellular material into the solution **(d)**

II.6.3. Experimental designs OO-laurel leaves

Table IX: Fully coded BDD and responses obtained from using UAM of OO-laurel

Run	Temperature (°C)	Time (min)	Ratio (%)	TPC (mg GAE/kg)	Predicted TPC (mg GAE/kg)
−−0	20	15	12,5	722,24	708,86
−+0	20	60	12,5	1080,72	1014,21
+−0	45	15	12,5	1036,34	1002,85
++0	45	60	12,5	950,18	963,562
0−−	32,5	15	5	902,58	889,77
0−+	32,5	15	20	818,75	878,421
0+−	32,5	60	5	1106,13	1066,45
0++	32,5	60	20	954,979	967,783
−0−	20	37,5	5	1173,73	1199,91
+0−	45	37,5	5	1004,14	980,44
−0+	20	37,5	20	790,05	803,75
+0+	45	37,5	20	1292,76	1266,57
000	32,5	37,5	12,5	895,52	906,07
000	32,5	37,5	12,5	959,63	906,07
000	32,5	37,5	12,5	913,060	906,07

- Based on the results of this study, the TP content of the enriched oil varies from 708, 86 mg GAE/ kg oil to 1266, 57 mg GAE/ kg oil, which confirms the influence of the selected parameters (time, temperature and ratio), on the degree of enrichment of the oil by the polyphenols of laurel leaves.

a. Analysis of the variance and validation of the model

An ANOVA has been realized on the results obtained **(Tab.X).** This analysis allow to test the relevance of the variables involved in the studied model and to present graphically the importance of each factor on response.

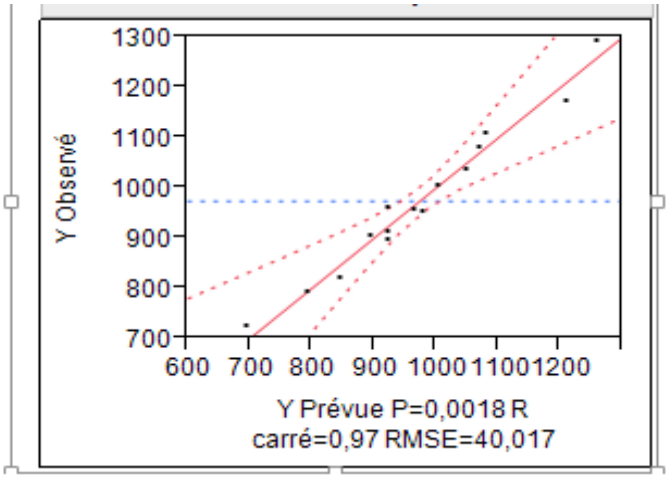


Figure 28: Comparison between predicted values of TPC and experimental values.

Table X: ANOVA TPC obtained in the BBD (OO-laurel)

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Linear effect					
A : Température	33370,55	1		20,8392	**0,0060**
B : time	46832,46	1		29,2458	**0,0029**
C : Ratio	13617,17	1		8,5036	**0,0332**
Interaction effect					
BC	1133,13	1		0,7076	0,4386
AB	49425,93	1		30,8654	**0,0026**
AC	112994,08	1		70,5623	**0,0004**
Quadratic effect					
AA	19194,04	1		11,9863	**0,0180**
BB	8318,85	1		5,1949	0,0716
CC	18265,69	1		11,4065	**0,0197**
Lack-of-fit	5811,4913	3	1973,16	1,7649	0,3816
Pure error	2869,4431	2	2195,1991	1097,60	
Total (corr.)	312568,42	14			

R^2= 97%, R^2 (adjusted for DF) = 0, 92%.

The analysis of variance data allowed us to deduce that the factors that preferentially affect enrichment of olive oil with antioxidants of the laurel leaves: temperature, time and ratio with a *P*-value of less than **0.05** at the **95%** confidence level. This is well illustrated in the Pareto diagram **(Figure 30).**

The overall fit efficiency is expressed by the regression coefficient R^2. In this study, the R2 value is **97%**, which means that only **3%** of the variations are not explained by the model. In addition, the value of the adjusted determination coefficient is high **(92%)** which confirms the high significance of the model. The value of lack of-fit was designed to determine whether the model chosen is valid for describing the observed data **(Achat et *al.*, 2012)**, so that this value is greater than **0.05** which confirmed validity of this plan.

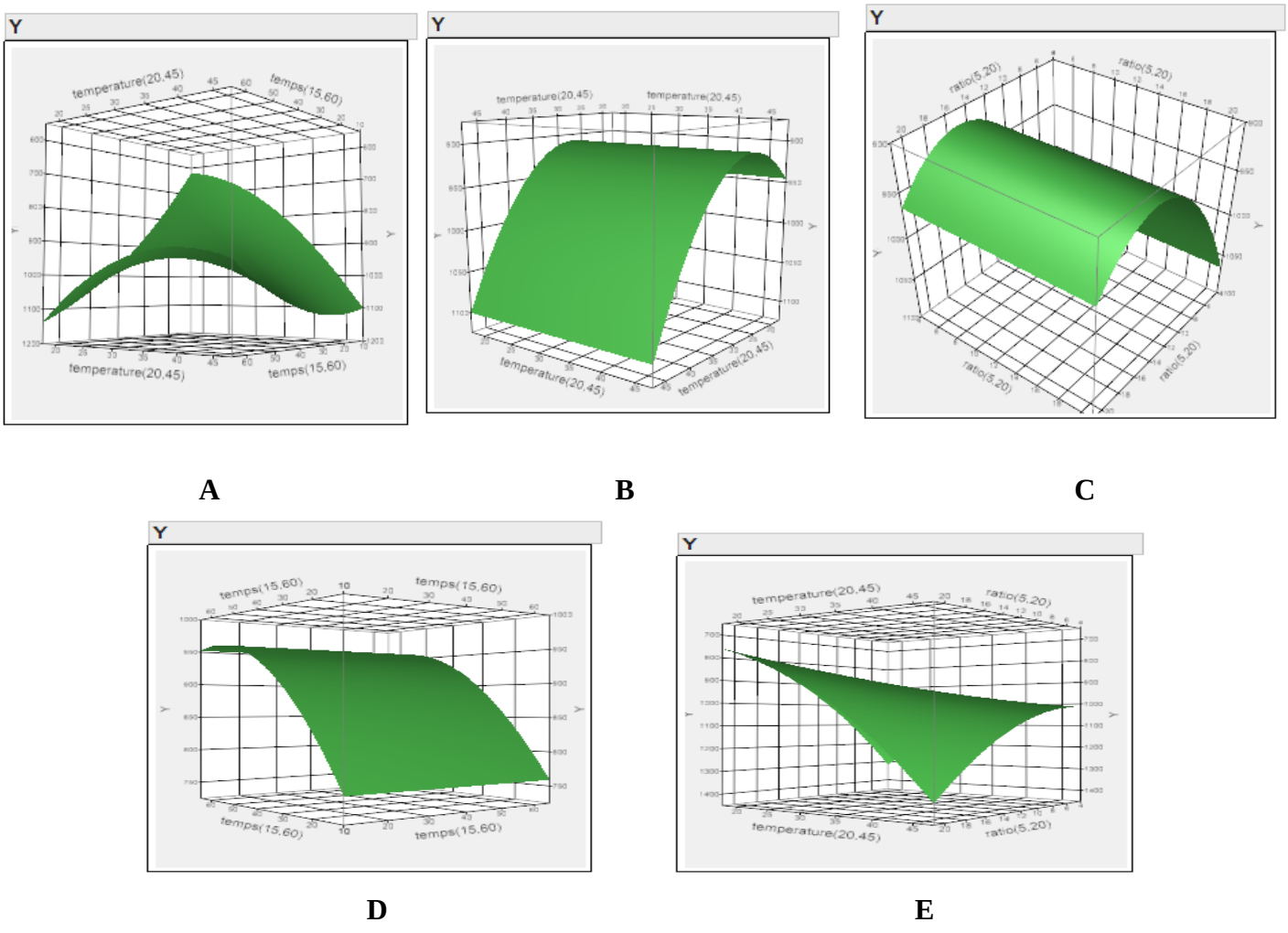


Figure 29: Response surfaces of the effect of different factors.

A: The interaction effects of temperature (20, 45) and time (15, 60).

B: Linear effect of temperature (20, 45).

C: Quadratic effect of ratio (5, 20).

D: Linear effect of time (15, 60).

E: The interaction effects of temperature (20, 45) and ratio (5, 20).

b. Surface response analysis

Once calculated, the total polyphenol concentrations are integrated into the JMP software which will estimate the importance of each parameter of the study. The Pareto diagram that allows to determine which parameters significantly influence TPC.

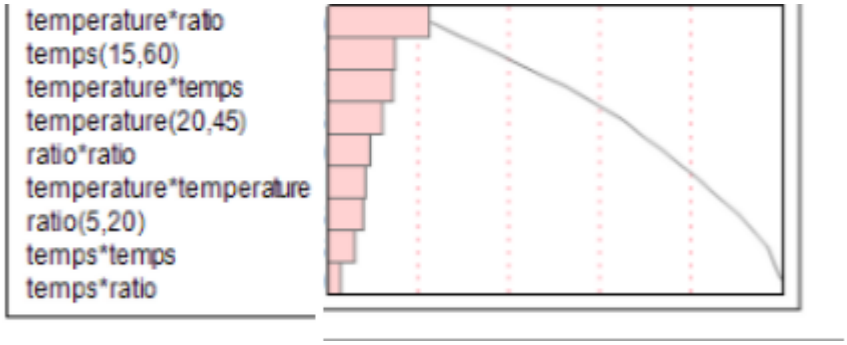


Figure 30: Pareto Diagram for TPC of OO-laurel.

The interaction effects of temperature and ratio has the most important influence on TPC, followed by the linear effects of time, then interaction of temperature and time. Whereas the cross product terms **(t, T*R)** show no significant effects. The lack of significance of these terms suggests the absence of interactions between variables in the studied zone. These results are listed in more details in the next figure:

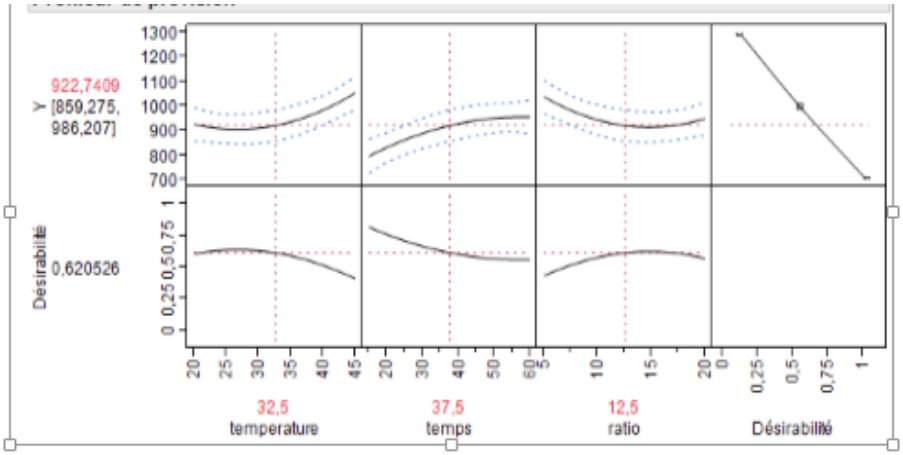


Figure 31: Graphical interpretation of results by JMP: Factor effects.

- Basing on the graph, the influence of temperature, time, and ratio on the polyphenols content gave a parabolic-like shape, where the peak value represents the optimum: 12.5%; 37.5 min and 32.5 °C, ratio, time and temperature respectively. Under these optimal conditions, the experimental value of TPC (928,196 ± 14,819 mg GAE / kg oil), was very close to the predicted value (922,740 mg GAE/ kg oil).

The predicted model was described by the following second-order polynomial equations:

TPC = 922, 74093 + 64, 585749*T + 79, 011812*t – 43, 75707*R – 111, 1597*T*t + 168, 07296* T*R + 69, 599838*T^2 + 72, 834611*R^2.

With:

T: Temperature;

t: Time ;

R : Ratio.

Hence, the response surface modeling could be applied effectively to predict the enrichment of olive oil by the compounds of laurel leaves.

Table XI: Optimal conditions of enrichment OO-laurel.

Variable	Response	
	Total Polyphenols	
Temperature (°C)	32,5	
Temps (min)	37,5	928,196 mg GAE/ kg of oil
Ratio (%)	12,5	

II.6.4. Comparison of UAM and CM (OO-laurel)

Figures 32, 33, 34 and 35, showed the same tendencies of results obtained in the enrichment of OO with peels, in terms TPC, TFC, reducing power and radical scavenging activity.

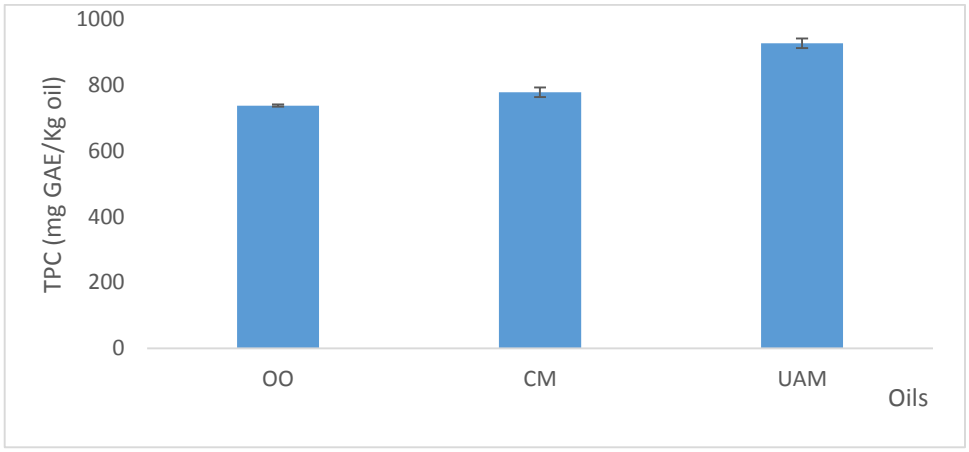


Figure 32: TPC of olive oil extracts before and after addition of laurel leaves

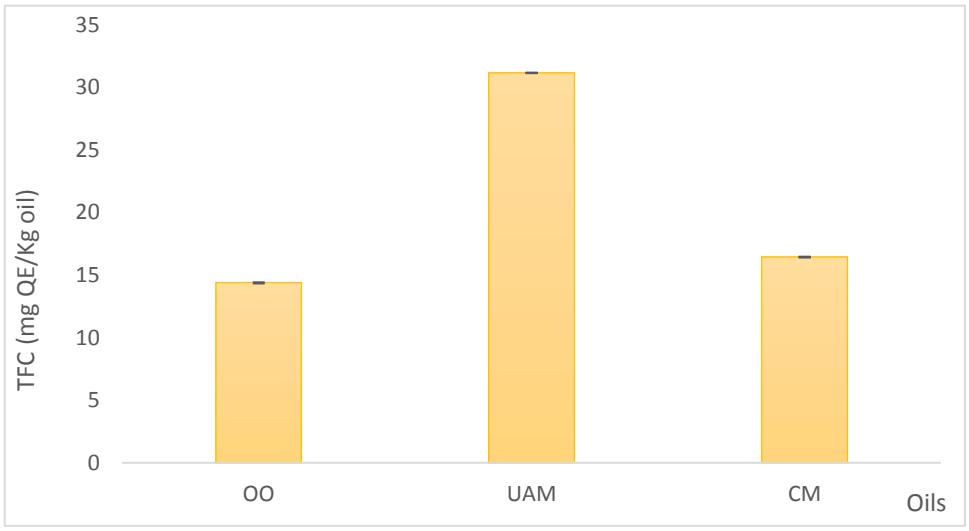


Figure 33: Effect of enrichment method on flavonoid content

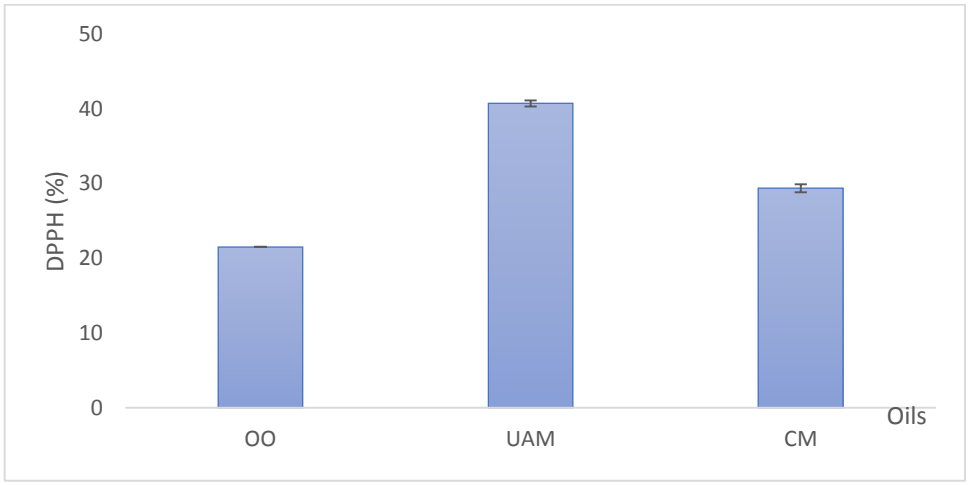


Figure 34: Antiradical capacity of the methanolic extracts of oils (UAM, CM).

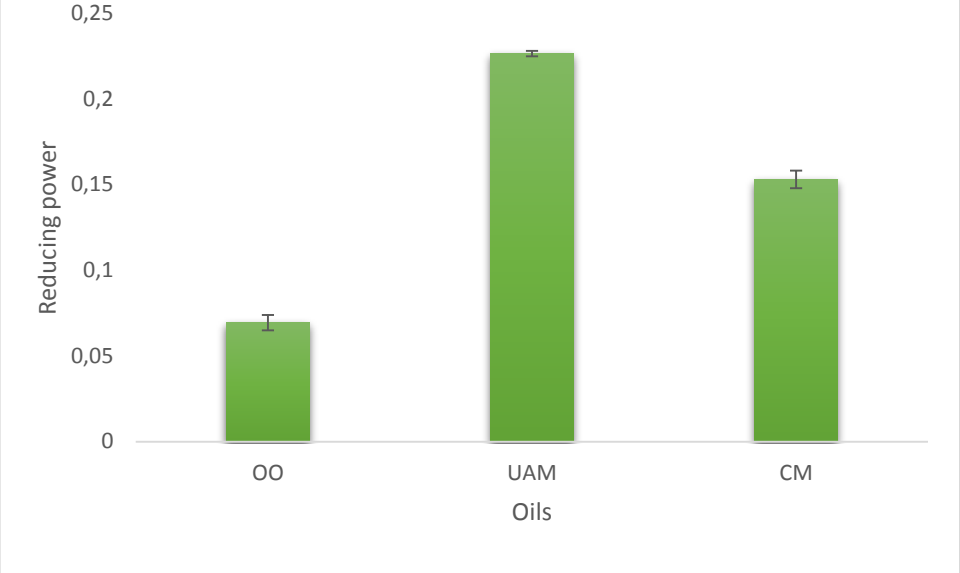


Figure 35: Reducing power of OO extracts before and after addition of laurel leaves.

UAM exhibited the highest TPC, TFC and antioxidant activity (DPPH° and reducing power), than CM. Indeed Conventional maceration procedures are either too long or demand too high temperature which causes a damage of the plant material. Ultrasound-assisted maceration is one of the novel procedures for the extraction of beneficial content in aromatic plants, and its use in organic solvents was already considered in the literature. **(Jović et *al*; 2018).**

- According to several authors, flavonoids are present in small quantities in olive oil **(Servili et *al*; 2004, Oliveras-Lopez et *al;* 2007).**

Conclusion

Conclusion

This study aims to extract the phenolic compounds of prickly pear peels, its seeds and laurel leaves assisted by ultrasound and optimize the conditions of extraction in order to work out a simple and effective extraction processes to employ them with fine and analytical industrialists.

A Box- Behnken plan was set up to study the effect of three independent variables: time (min), temperature (c°) and ratio (%) on the extraction of the total phenolic compounds in order to define the mathematical model allowing the optimization of the conditions of extraction.

Under these conditions, for prickly pear peels, TPC are estimated at **1057, 43 mg EAG / kg of oil**, a huge increase compared to that of virgin oil **(738 mg EAG / kg of oil),** likewise flavonoids which rose from **14, 39 mg EQ/ kg of oil** to **34,067 mg EQ /kg of oil,** reducing power which increase from **0,069 to 0,131.** The value of betalains content is estimated at **261 (mg/100g DM)** compared to **1, 19 (mg/100g DM)** contained in virgin oil**.** The DPPH increase from **21, 5 %** to **88.09 %**, a very important increase by adding virgin olive oil. For laurel leaves, TPC are estimated at **928, 19 mg EAG / kg of oil,** flavonoids with a value of **31,156 mg EQ /kg of oil,** reducing power which increase from **0,069 to 0, 226** and DPPH **62.69 %**.

The variance analysis for the effect of the factor on TPC for the prickly pear peels and laurel leaves in the case of ultrasound assisted extraction, gives coefficients of determination R2 of **0, 95** and **0, 97** respectively and the values of the coeffecients of determination adjusted (R adjusted) are about **0, 86** and **0, 92**. This analyze watch that the model is significant **<0, 0005**.

In conclusion, this modest work constitutes at the same time a valorization of under produced products of prickly pear (peels) and laurel leaves which are rich in phenolic compounds, have antioxidant properties and in addition, an improvement of the quality of refined oil while bringing more active ingredients to him.

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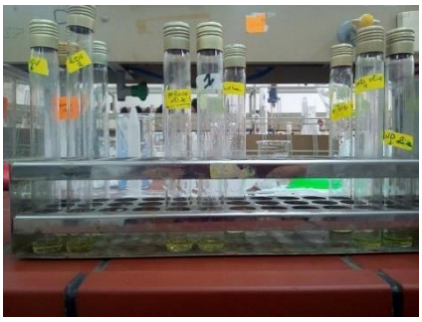
Appendix

Appendix

(a)

(b)

Training of 2 phases after methanol addition oily extracts for laurel leaves (a) and prickly pear peels (b)



Addition of FC reagent.

Addition of sodium carbonate

Abstract

In this study, an ultrasound extraction method was used to extract total polyphenols from prickly pears peels and laurel leaves.

In order to have the possibility to improve the nutritional value of the virgin oil by enriching it with the phenolic compounds of laurel leaves and prickly pear peels, an application of experimental designs is envisaged in order to optimize extraction conditions. The effect of independent variables (time, temperature and ratio) on TPC levels was evaluated using the RMS surface response methodology.

Variance analysis confirmed that the contribution of a quadratic model and the effect of interactions are significant for the combination response (a temperature of 32, 5 ° C; an extraction time of 37, 5 min and a ratio of 12, 5%). In addition, DPPH tests performed; the reducing power; flavonoids, as well as betalains (for peel) have confirmed its richness in antioxidants.

Key Word: enrichment, experimental design, surface response, enrichment, ultrasound method, phenolic compounds.

Résumé

Dans cette étude, une méthode d'extraction par ultrasons a été utilisée pour l'extraction des polyphénols totaux à partir de la pelure de la figue de barbarie et des feuilles de laurier.

Afin d'avoir la possibilité d'améliorer la valeur nutritionnelle de l'huile vierge en l'enrichissant par les composés phénoliques des feuilles de laurier et de la pelure de la figue de barbarie, 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 et le DPPH a été évalué 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 de 32,5C° ; un temps d'extraction 37,5 min et un ratio de 12,5%. En outre, les tests du DPPH réalisés ; le pouvoir réducteur ; les flavonoïdes, ainsi que les betalains (pour la pelure) ont confirmés sa richesse en antioxydants.