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THÈSE

EN VUE DE L'OBTENTION DU DIPLOME DE DOCTORAT

Domaine : Sciences de la Nature et de la Vie **Filière :** Sciences Biologiques
Spécialité : Agro-ressources, Bioprocédés et Sciences Alimentaires

Présentée par
GUEMGHAR Menana

Thème

Valorisation des déchets d'artichauts (*Cynara scolimus*): Extraction des substances actives et applications alimentaires

Soutenue le : 20/02/2021 **Devant le Jury composé de :**

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Faculty of Natural and Life Sciences
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THESIS

IN ORDER TO OBTAIN THE DIPLOMA OF DOCTORATE

Field: Natural and Life Sciences **Sector:** Biological Sciences
Speciality: Agro-resources, Bioprocesses and Food Sciences

Presented by
GUEMGHAR Menana

Theme

Valorization of waste artichoke *(Cynara scolymus):* Extraction of active substances and food applications

Supported on: 20/02/2021 **In front of the Jury composed by:**

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Dedication

A dream that is being fulfilled thanks to God the Almighty, this work consisting of the doctoral thesis is finally finished, I dedicate it to the people who are very dear to me:

In memory of my dearest father, may his soul rest well in heaven, this work is the fruit of his advice which will remain forever engraved in my heart and mind as well as in the memory of my very dear sister Saida.

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1. **PHENOLIC COMPOUNDS FROM ARTICHOKE (Cynara scolymus L.) BYPRODUCTS: OPTIMIZATION OF MICROWAVE ASSISTED EXTRACTION AND ENRICHMENT OF TABLE OIL.** MENANA GUEMGHAR[1], HOCINE REMINI1,[2], NADIA BOUAOUDIA-MADI1,[3], KHOKHA MOUHOUBI[1], KHODIR MADANI1,4, LILA BOULEKBACHE-MAKHLOUF[1]. The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology (2020), 44(1), 193-211
2. **.Valorization of *Cynara scolymus L.* (artichoke) waste for the recovery of antioxidants: optimization of ultrasound- and microwave-assisted extraction and food application.** Menana Guemghar[1], Lila Boulekbache-Makhlouf[1], Federica Pellati[2], Artur M.S. Silva[3], Khodir Madani[1], Susana M. Cardoso[3] (Submitted)

Participation in scientific events

Communications

1. **Menana GUEMGHAR[a]**,, Giorgia Spigno[b], khodir MADANI[a], Lila BOULEKBACHE-MAKHLOUF[a] ; The phenolic profile and dietary fiber distribution in artichoke by-products (stem) .15/16 DEC. 2018 –CHEMINI- Béjaja- ALGERIA.
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3. **Menana GUEMGHAR**, Lila BOULEKBACHE-MAKHLOUF, Khodir MADANI and Susana MARIA DE ALMEIDA CARDOSO ; Enrichment of a vegetable oil by antioxidants from artichoke byproducts (Stem) ; International Congress ; The Meetings of Agriculture and Biology (RAB) 5 to 7 May, 2018 Constantine.
4. **Menana GUEMGHAR**, Lila BOULEKBACHE-MAKHLOUF, Susana MARIA DE ALMEIDA CARDOSO , khodir MADANI ; Valorization of artichoke waste generated

during industrial agro processing for the recovery of polyphenols : comparison of microwave and ultrasound assisted extactions to conventional method and evaluation of tiers anti-oxidante activity ; 5th Days of the Young Researcher Constantine 23-24 April 2018

5. **Menana GUEMGHAR**, Boulekbache-Makhlouf Lila, Remini Hocinea, Belhabib-Mamou Kahina , Madani khodir ; Valorisation of artichoks's by- products: Drying kinetics of artichoke waste generated during industrial agro processing ; 29éme congrés international des sciences biologiques et de la biotechnologie de l'ATSB, Hotel Mouradi Palace, 26th to 29th of March 2018, Sousse, Tunisia
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Organizations

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List of abbreviations

ABTS	2, 2'-9-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	Analysis Of Variance
Aw	Activity of water
BBD	Box–Behnken Design
C.V.	Coefficient of Variation
CSE	Conventional Solvent extraction
DF	Degree of Freedom
d.d.l	degrees of freedom
DO	Optical density
DPPH	2,2-Diphenyl-picrylhydrazyl
DW	dry weight
EVOO:	Extra Virgin Olive Oil
EEVOO:	Enriched Extra Virgin Olive Oil
F–value	Fisher value
FAO	Food and Agricultural Organization
JMP	John's Macintosh Project, pronounced "jump"
HPLC**:**	High Performance Liquid Chromatography
TPC	Total Phenolic Compounds
GAE	Gallic Acid Equivalent
M	Molar
MAE	Microwave Assisted Extraction
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
Na_2CO_3	sodium carbonate
pH	potential of Hydrogen
r	correlation coefficient

RSM	Response Surface Methodology
RMSE	Root Mean Square Error
R^2	Coefficient of determination
$R^2_{adjusted}$	Adjusted coefficient of determination
pH	hydrogen potential
ROS	reactive oxygen species
SS	summon square
TC	condensed tannins
UV	ultra-violet
v/v	volume/ volume
W	Watt

List of tables

Tables in annexes

List of figures

Figures in annexes

Table of contents

Table of contents

Chapter III: Microwave Assisted Extraction (MAE)

Chapter IV: General Information on Sunflower oil

Second Part: Experimental part

(Part II-A-)

Materials and methods

"Section I"

- ***The optimization and comparison of phenolic compounds extraction from artichoke (Cynara scolymus L.) waste: The determination of the optimal MAE conditions, and the characterization of the stem extracts by HPLC analysis***

Results and discussion (Part II-A-)

"Section I"

Materials and methods (Part II-A-)

"Section II"

- ***The determination of dietary fiber distribution in different particles size of (Cynara scolymus L.) stem.***

Results and discussion (Part II-A-)

"Section II"

(Part II-B-)

The Enrichment of a vegetable oil by antioxidants (the optimal MAE extract) of artichoke by-products, and the characterization of both enriched and control oils by HPLC analysis

Materials and methods

Results and discussion

(Part II-B-)

General introduction

It is estimated that about 1.3 billion of food wastes are produced per year, which cause important problems for the industry and the environment (Arshadi, Attard, Lukasik, Brncic, da Costa Lopes, Finell, et al., 2016; Fregapane, Guisantes-Batan, Ojeda-Amador, & Salvador, 2020), such as the collection cost, industrial processing and environmental pollution. Indeed, the magnitude of loss and waste is too large to be ignored, so one-third of the edible parts of global food produced for human consumption is estimated to be lost or wasted (Lopez Barrera & Hertel, 2020). Agro-industrial wastes are rich sources of bioactifs compounds, which have been widely studied due to their potential health-promoting properties (Castro-Vargas, Ballesteros Vivas, Ortega Barbosa, Morantes Medina, Aristizabal Gutierrez, & Parada-Alfonso, 2019; Shahidi, Varatharajan, Oh, & Peng, 2019). Currently, the exploitation of theses wastes and the use of phenolic antioxidant-rich plant extract as food additives and/or nutraceutical have both been of increasing interest. This is because wastes of plant origin often contain natural antioxidants which are much safer than synthetic antioxidants and constitute a large source of valuable compounds (Yu, Yang, Sato, Yamaguchi, Nakano, & Xi, 2017).

Several studies demonstrated different phenolic compounds and antioxidant activity from food plant by-products such as grape pomace and seeds from processed winery (Bozan & Temelli, 2008; Lafka, Sinanoglou, & Lazos, 2007), pomegranate marc (Qu, Pan, & Ma, 2010), soybean (Tyug, Prasad, & Ismail, 2010) and orange peel (Khan, Abert-Vian, Fabiano-Tixier, Dangles, & Chemat, 2010) and there has been a significant data showing that these by-products could be used to produce innovative foods as they might promote human health (Djaoud, Boulekbache-Makhlouf, Yahia, Mansouri, Mansouri, Madani, et al., 2020). Indeed, the daily intake of phenolic compounds is very variable due to different proportion of the different food groups and individual foods of our diet. Thus, a good strategy to ensure an optimal intake of polyphenols through the diet would be to make available enriched oils with well-known bioactive polyphenols. Different sources of biologically active substances can be potentially used to enrich edible oils (Dairi, Carbonneau, Galeano-Diaz, Remini, Dahmoune, Aoun, et al., 2017). Indeed, the development of these functional oils may help in prevention of chronic diseases (such as cardiovascular diseases, immune frailty, ageing disorders and degenerative diseases) (Torrent, Arce Menocal, López Coello, & Ávila González, 2019) and improving the quality of life for many consumers reducing health care costs (Fregapane, Guisantes-Batan, Ojeda-Amador, & Salvador, 2020).

The first step for both analysis and exploitation of plant bioactive constituents is their extraction from the cellular matrix. The “ideal” extraction method should be quantitative, non-destructive, and time saving (Dahmoune, Boulekbache, Moussi, Aoun, Spigno, & Madani,

2013). The extraction of phenolic compounds requires special care, because they are easily oxidized and rapidly degraded by light. This consists in the extraction procedures design that use environmental friendly solvents, classified as minimal or no harm upon ecosystems or the environment (Płotka-Wasylka, Rutkowska, Owczarek, Tobiszewski, & Namieśnik, 2017), and renewable products, reduce the consumption of energy and have a suitable extract in terms of safety and other quality parameters as result (Chemat, Vian, & Cravotto, 2012). Indeed, this method is used for the production of extracts rich in phenolic antioxidants from *Olea europaea* L. and *Cynara scolymus* L. matrices (Płotka-Wasylka, Rutkowska, Owczarek, Tobiszewski, & Namieśnik, 2017).

Recently, different novel and emerging technologies for green extraction such as High Hydrostatic Pressures (HHP), Ultrasound (US), Pulsed Electric Fields (PEF) and Microwaves (MW) are being increasingly used (Deng, Zinoviadou, Galanakis, Orlien, Grimi, Vorobiev, et al., 2015; Kyriakopoulou, Papadaki, & Krokida, 2015). In this regard, MAE appears to be one of the best methods to extract phenolic compounds due to the special microwave/matter interactions and the very rapid extraction time (Setyaningsih, Saputro, Palma, & Barroso, 2015). The MAE system rapidly generates heat and this characteristic results in a shorter extraction time, efficient extraction, automation, and reduction of organic solvent consumption (Spigno & De Faveri, 2009).

Different chemical substances absorb microwaves to different extents and this behaviour makes MAE an efficient method for extraction and, more importantly, it makes it possible to selectively extract target compounds from complex food matrices (Eskilsson & Björklund, 2000).

Extraction technique, temperature, time, type and solvent concentration, solvent to solid ratio, and extraction cycles are some of the factors which individually and/or in combination affect the extraction efficiency (Karabegović, Stojičević, Veličković, Nikolić, & Lazić, 2013). Optimization of these parameters is very important to obtain high extraction yields. Response surface methodology (RSM) is the most commonly used for development, improvement and optimization of extraction processes (Simić, Rajković, Stojičević, Veličković, Nikolić, Lazić, et al., 2016). Moreover RSM is an efficacious mathematical and statistical technique for simultaneously evaluating the interaction of several experimental parameters (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Izadiyan & Hemmateenejad, 2016).

Globe artichoke (*Cynara scolymus L.*) is a perennial plant originating from the Mediterranean region and grown for its edible flower buds. The most recent statistics about globe artichoke in the world show that the most important producer country is Italy with more

than 45,000 ha and ~440,000 t year. Traditionally globe artichoke is cultivated in Mediterranean countries, with more than 76% of the world's globe artichoke growing areas (Riahi, Nicoletto, Bouzaein, Haj Ibrahim, Ghezal, Sambo, et al., 2019). It is extensively cultivated in the Mediterranean region, constituting the 85 % of world production (Romani, Scardigli, & Pinelli, 2016; Ruiz-Aceituno, Garcia-Sarrio, Alonso-Rodriguez, Ramos, & Sanz, 2016). The edible parts of the artichoke are large inflorescences, collected in the early stages of their development, representing about 30–40 % of its tare weight. Since only the central part of the inflorescence is being consumed, the ratio "edible fraction/total biomass" is rather low (less than 15–20 % of the total plant biomass) (Angelov, Georgieva, Boyadzhieva, & Boyadzhiev, 2015). During the industrial processing of artichokes, about 80 –85 % of the total plant biomass is discarded and turned into a solid waste (Zuorro, Maffei, & Lavecchia, 2016). This material consists mainly of the stems and the external parts of the flowers, commonly known as bracts, which are unsuitable for human consumption. The valorisation of both residues would improve the industrial process both economically and environmentally and will allow a massive reduction in biomass wastes (Zuorro, 2014). However, an analysis of its composition shows that artichoke waste is a very rich source of phenolics (Gaafar & Salama, 2013), that exhibit a wide range of beneficial health effects, such as: anti-allergenic, anti-inflammatory, antioxidant, antimicrobial, antithrombotic, cardio protective and vasodilatory effects. Several beneficial effects of polyphenols are mainly due to their antioxidant activity (Yolmeh, Habibi Najafi, & Farhoosh, 2014).

The enrichment of lipid food by natural antioxidant was recently developed as a new way of phytochemical potential application. The enrichment of extra virgin olive oil (EVOO) by green tea phenolic compounds improved significantly the antiatherogenic properties of olive oil resulting in significant attenuation of atherosclerosis development (Rosenblat, Volkova, Coleman, Almagor, & Aviram, 2008). Moreover, in a mixture of phenolic compounds, synergistic or antagonistic effects may occur and this could modulate the total antioxidant activity of the whole food. Subsequently, it is very important to take into account all these interactions when designing a functional food (Palafox-Carlos, Gil-Chávez, Sotelo-Mundo, Namiesnik, Gorinstein, & González-Aguilar, 2012).

To the best of our knowledge, no literature report exists on the optimization of MAE procedure for the extraction of total phenolic compounds (TPC) from artichoke stem. Consequently, the objectives of the present study are: the optimization of the extraction of TPC from stem by using green technology (MAE) and their valorisation by enrichment application of table oil. Moreover, the optimized extracts were characterized regarding their phenolic composition and antioxidant activity, also comparing to those of a reference conventional

solvent extraction procedure (CSE). At last, the feasibility of using archickoke MAE extracts as antioxidant ingredients was tested by its incorporation in a sunflower oil, with attested increment of the phenolic profile and antioxidant abilities of the final products and dietary fiber distribution from artichoke (*Cynara scolymus L.*) waste.

To this end, the work of this thesis will be presented in two parts:

-The first includes the bibliographic study, which is subdivided into four chapters:

*General information on artichoke (*Cynara scolymus L)* and its by-products,

* Phenolic compounds: Chemistry, reactivity and antioxidant activity evaluation methods

*General information on Microwave Assisted Extraction (MAE),

*General information on sunflower oil.

-The second part illustrates the experimental part and is in turn subdivided into two parts:

*Part II.A. Which is subdivided into two sections:

- The optimization and comparison of phenolic compounds extraction from artichoke (*Cynara scolymus L.*) waste: The determination of the optimal MAE conditions, and the characterization of the stem extracts and by HPLC analysis
- The determination of dietary fiber distribution in different particles size of (*Cynara scolymus L.*) stem.

*Part II.B. The Enrichment of a vegetable oil by antioxidants (the optimal MAE extract) of artichoke by-products, and the characterization of both enriched and control oils by HPLC analysis.

Finally, our manuscript is punctuated by a general conclusion and possible perspectives

First part: Literature review

Chapter I: General Information on Artichoke and its by-products

I.General information on artichoke

I.1. Definition, history and habitat

The artichoke has been used for both nutritional and medicinal uses for thousands of years. The first reports of artichokes came from Theophrastus around 300 B.C. The Greeks, Romans, and even the Egyptians incorporated the artichoke into daily life. In Rome, it was staple menu item at feasts. They considered it be an aphrodisiac and even claimed that it could be effective in securing the birth of boys. After the fall of the Roman Empire, the population of artichokes dwindled and they became a rarity. However, they again became popular during the Renaissance when the Strozzi family brought them from Florence to Naples. The cultivation of the artichoke throughout Europe did not occur until the fifteenth century. Artichokes first appeared in the United States when French immigrants brought them over in 180 (Foury, 1997)

Cynara scolymus (Asteraceae)(table I) is a native plant of the Mediterranean region (southern Europe and North Africa) and is also seen in subtropical climates such as Brazil where it is known as artichoke. The C. scolymus is a plant rich in nutritious and possesses numerous medicinal properties. In other words, it is not only cultivated all around the world because of being a healthy tasty; rather, it is viewd also as an herbal drug (Christaki, Bonos, & Florou-Paneri, 2012). Currently the artichoke is cultivated in different countries such as the United States (mainly in California), South America, North Africa, Near East Turkey, Iran and China. Artichoke is consumed as a fresh, frozen, or canned vegetable (Sadeghzade Namavar, Amiri Chayjan, Amiri Parian, & Zolfigol, 2018) . Globe artichoke is a perennial, rosette plant, widely cultivated for its large fleshy head or capitula (immature flowers) (Figure 1) which represent 30-40% of the fresh weight (FW). The edible parts of the artichoke are the tender inner leaves (bracts) and the receptacle commonly known as “heart” that constitute nearly 35-55% of the FW of the head (Turkiewicz, Wojdyło, Tkacz, Nowicka, & Hernández, 2019).The artichoke crop may last six or more years, reaching the maximum productivity in the third year.

Atichoke Leaf Extracts (ALEs) are extensively used alone or in association with other herbs to prepare herbal teas (Salem, Affes, Ksouda, Dhouibi, Sahnoun, Hammami, et al., 2015). Polyphenolic compositions, as an active component of this plant exist significantly from its leaf rather than its heads. Biological composition of an extract of artichoke leaf shows a low fat content and a high amount of minerals (phosphorus, sodium and potassium), vitamin C, fibers, polyphenols, flavones, inulin and caffeoylquinic acid derivatives .Due to anti-cancer properties of these compounds, they play an important role in human nutrition. Hence researcher suggest taking into account the‘‘functionalization’’ of foodstuffs by adding artichoke by-product extracts. Regarding the demands of modern therapeutic operation, ease of standardization and handling, and producing a homogenized products make herbal dried extracts suitable for medicinal applications(Ross & Preedy, 2014).

Although it has become a popular vegetable in the United States, the Globe Artichoke was actually thought to have been brought over from Europe. It is now cultivated all over the world including the Mediterranean, France, Italy, and Spain. Today, California provides almost 100% of the artichokes used and consumed by citizens of the United States. It grows best in moist, sandy environments with full exposure to the sun. They prefer ground cover that can drain easily. Because of the artichokes durability, it is hardy to temperatures below freezing.Some plants are able to grow in more extreme.

I.2 Classification and botanical description

I.2.1 Classification

Table I Taxonomic classification of artichoke

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons

Subclass	Asteridae
Order	Asterales
Family	Asteraceae / Compositae – Aster family
Genus	*Cynara* L. – cynara
Species	*Cynara scolymus* L. – globe artichoke

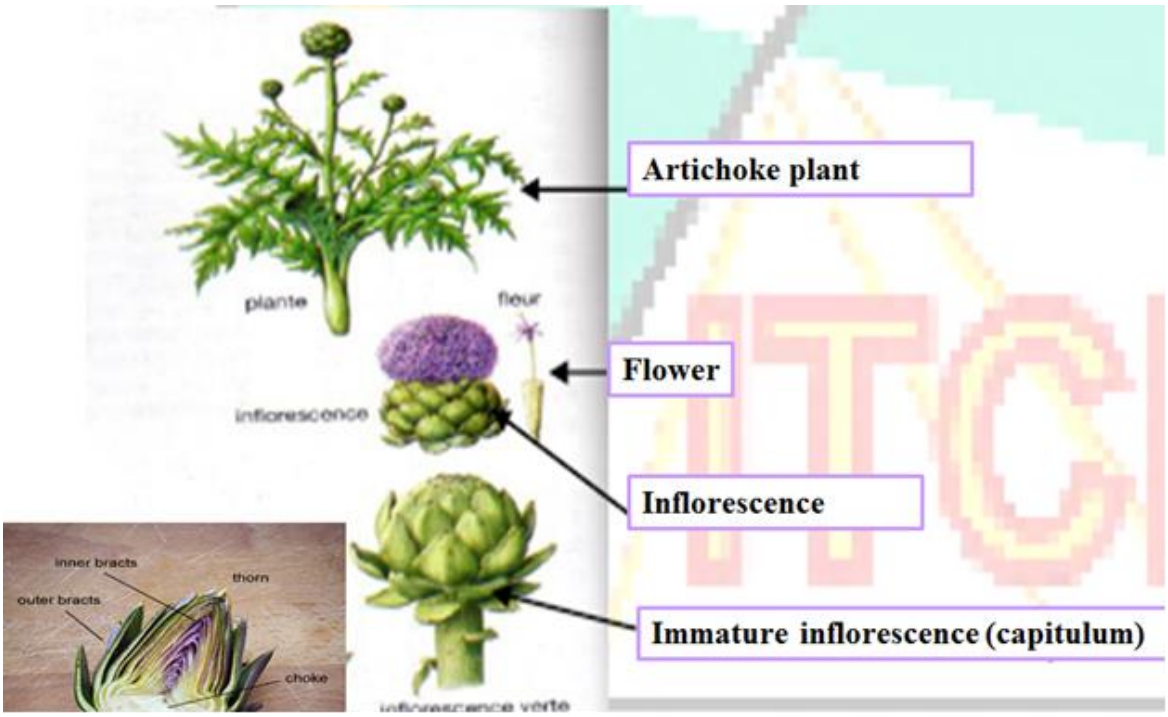

Figure.1. Artichoke plant (*Cynara scolymus L.*)

I.3. Artichoke by-products; phytochemicals and biological functions

Non-edible parts of *artichoke* represent valuable sources of minerals and phytochemicals, where green parts of wild cardoon are nutritive as edible flower heads and contain essential polyunsaturated fatty acid (PUFA) *i.e.*, α-linolenic acid (18:3–3) and tocopherols, in addition to phenolic acids (Chihoub, Dias, Barros, Calhelha, Alves, Harzallah-Skhiri, et al., 2019). PUFA and tocopherols contents were at 1.11 and 9.6 mg/100 g dry weight higher than biowastes of turnip and radish. Additionally, cardoon seeds are enriched in fat (17–23 g/100 g), protein (25–30 g/100 g), fiber (20–27 g/100 g), in addition to macro and microelements (i.e., K, Ca, Mg, Fe ≫ Na). Particularly, seed oil is highly nutritive and

represents a rich source of unsaturated and saturated fatty acids. The unsaturated fatty acids are mainly linoleic and oleic acids, amounting for 44 and 43 g/100 g of its oil composition, respectively (Barracosa, Barracosa, & Pires, 2019).

In contrast saturated fatty acids are present at lower levels represented by palmitic and stearic acid at 10 and 3 g/100 g, respectively (Petropoulos, Pereira, Tzortzakis, Barros, & Ferreira, 2018). These chemicals exhibit beneficial health-promoting activities, suggesting that artichoke non-edible parts have potential as a nutraceutical and suitable oil for human consumption. Likewise, Dabbou, et al. investigated the phytochemical profile of globe artichoke cultivars by-products grown in Tunisia. They found that bracts are enriched in phenolic compounds (*i.e.*, flavonoids and anthocyanins) and terpenoids (*i.e.*, sesquiterpene hydrocarbons and non-terpene derivatives) (Dabbou, Dabbou, Flamini, Pandino, Gasco, & Helal, 2016). The leaves represent a good reservoir of phenolic acids, flavonoids, and sesquiterpene lactones (STLs) (Rouphael et al., 2016). Furthermore, another chemical of value, present in large amounts in artichoke, is inulin found in its stem, bract, and root, and amounting for 5–7 g/100 g of globe artichoke fresh weight (Leroy et al., 2010; Raccuia). Inulin is a soluble dietary fiber that is found in a variety of fruits and vegetables that are converted by colon bacteria into short-chain fatty acids (SCFAs) known to nourish colon cells and stimulating the immune system concurrent with enhanced calcium absorption and lowered serum lipids) (Fissore, Santo Domingo, Gerschenson, & Giannuzzi, 2015). Table II summarizes phytoconstituents and major biological effects in artichoke bi-products.

Table .II. Selected phytochemicals and biological activities of non-edible parts of artichoke extracts.(Zayed & Farag, 2020)

Biogenic source	Phytochemicals	Biological activities	Ref.
Globe artichoke (C. cardunculus L. var. Scolymus)			
Leaf	Sesquiterpene lactones (*i.e.*, cynaropicrin and grosheimin) Phenolics (i.e., chlorogenic acid andcynarine) and	-Anti-inflammatory, -Antiviral Antimicrobial	(Eljounaidi et al., 2015; Elsebai,Abass, et al., 2016) Zhu, Zhang, and Lo(2004) (Ben Salem et al.,

	flavonoids (i.e.,cynaroside and scolymoside)	-Hepatoprotective and hypolipidemic	2019; Speroni et al.,2003)
	Flavonoids, Hydroxycinnamic acids, tyrosols, and lignans	-Antioxidant	Rouphael et al. (2016)
	Phenolic acids (i.e.,hydroxytyrosol verbascoside) and flavonoids (i.e.,apigenin-7-Oglucoside)	-Anti-hyperglycemic -Antioxidant and - Hypolipidemic Hepatoprotective	Ben Salem, Ben Abdallah Kolsi et al. (2017) Ahmadi, Heidarian, and Ghatreh-Samani (2019)
Bract	Polyphenolic compounds (i.e.,monoand di-caffeoylquinic acids	Anti-diabetic	Turkiewicz, Wojdyło, Tkacz, Nowicka, and Hernandez (2019)
Cultivated cardoon (C. cardunculus L. var. Altilis)			
Leaf	Lipophilic compounds (i.e., cynaropicrin and taraxasteryl acetate) Caffeoylquinic acids, luteolin/apigenin derivatives, and cynaropicrin	Anti-proliferative Antibacterial	Ramos et al. (2016) Scavo et al. (2019)
Wild cardoon (C. cardunculus L. var. Sylvestris)			
Leaf	Phenolic acids (i.e., hydroxy cinnamic acid derivatives) and flavonoids (i.e., apigenin-7-Oglucoside, cymaroside and scolymoside) compounds	-Anti-HCV	(Elsebai, Abass, et al., 2016; Elsebai et al., 2015; Farag et al., 2013)
Seed		-Antioxidant	Durazzo et al. (2013)

I.4. Artichoke biowastes and food industry

The concept of biowastes valorization is of increasing interest especially that it can be applied to biorefinery platforms generating sustainable and value-added by-products, (*i.e.*, biofuels, bio-materials, biostimulants, fine chemicals, and other value-added components) and can help solve agri-food industry problems (Zayed & Farag, 2020). The attention towards profitable reuse of biowastes aims to recover the well-known valuable compounds of artichoke before its use for value-added purposes that provide a close-to-zero-waste biorefinery. Recent investigations have focused on developing new techniques to explore potential applications of the bioactive compounds obtained from food biowastes and its valorization to even other novel

relevant products. Examples include the use of citrus peel for the production of pectin, enzymes, dietary fibers, and food additives used in food, cosmetic, and pharmaceutical industries (. Also, the production of biofuels, biopolymers, biosurfactants, and chemicals are produced *via* fermentation and enzyme processing technologies of date palm biowastes (Zema, Calabrò, Folino, Tamburino, Zappia, & Zimbone, 2018)

The increase in global demands for dairy products, the high price of calf rennet, and religious and genetically engineered food concerns have driven investigations to develop alternatives from plant resources (Esposito, Di Pierro, Dejonghe, Mariniello, & Porta, 2016). Wild cardoon flower extracts are reported to be used as a vegetable alternative and milk coagulants for the manufacture of dairy products (Almeida & Simões, 2018), and globe artichoke flower heads represents a source of milk clotting aspartic proteases (EC 3.4.23) also named cardosins/cyprosins or cynarases (*i.e*., cardosin A and cardosin B) (Sidrach, García-Cánovas, Tudela, & Rodríguez-López, 2005). Inulin derived from root, stem, and bracts has been employed as a fat substitute in chicken sausages formulations (Alaei, Hojjatoleslamy, & Hashemi Dehkordi, 2018) and has been applied in low-fat dairy products *i.e*., yogurt (Faustino, Veiga, Sousa, Costa, Silva, & Pintado, 2019). Moreover, globe artichoke total phenolics content in non-edible receptacles, with chlorogenic acid, *p*-coumaric acid, and ferulic acid detected as major antioxidant phenolic acids. With the addition of 0.25 g of artichoke spikes and bracts, an increase of the oxidation latency was observed by 72% and 16%, respectively for 5 g canola oil revealing a remarkable preservative action of artichoke. This could be further considered for mitigating oil autoxidation and rancidity, with comparable activity to the 39% inhibition for the synthetic chemical butylated hydroxytoluene (BHT) at a dose of 0.001 g/5 g canola oil (Claus, Maruyama, Palombini, Montanher, Bonafé, Junior, et al., 2015; Faustino, Veiga, Sousa, Costa, Silva, & Pintado, 2019). Furthermore, dietary fibers (*i.e.*, inulin and pectin) derived from globe artichoke stem and bract are of high value as bakery and jam ingredients. Incorporation of fiber concentrate with wheat flour results in improved dough rheological properties, (*i.e*., water absorption, stability, extensibility, tenacity, and softening). Moreover, such a bread product showed a comparable specific volume and improved shelf life compared with a product free from fibers (Boubaker, Omri, Blecker, & Bouzouita, 2016). Pectin derived from globe artichoke bracts, leaves, and stems (20 g/100 g) has additional industrial applications in the food industry as gelling agent and prebiotics for good functioning gut flora (Sabater, Corzo, Olano, & Montilla, 2018) , and whether it can be incorporated in the manufacture of probiotic

nutraceuticals has yet to be performed. For a simple overview, the different constituents and applications are summarized in Figure. 2.

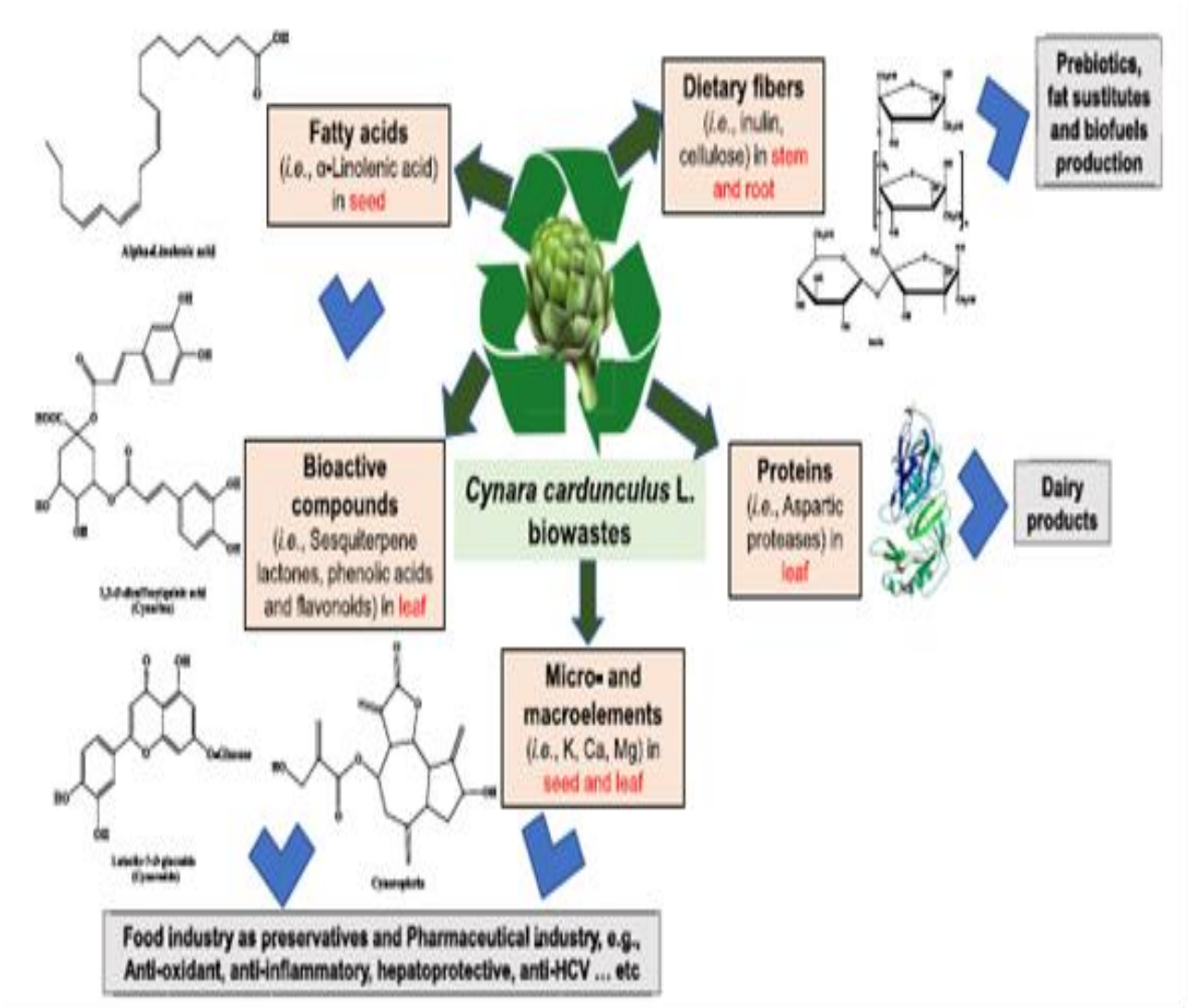

Figure. 2. Valorization of *C. cardunculus* L. and its by-products: chemical composition, value-added and applications (Zayed & Farag, 2020)

Chapter II: Phenolic compounds: Chemistry, reactivity and antioxidant activity evaluation methods

II.Phenolic compounds: chemical structure and properties

II.1 Definition

Phenolic compounds are a group of aromatic secondary plant metabolites and they are one of the largest groups of plants metabolits. More than 8000 polyphenolic compounds have been identified in various plant species. These compounds are divided into two main categories, the flavonoids and non-flavonoids, based on the number of phenol rings and the way in which these rings interact metabolites (Han, Shen, & Lou, 2007).

They arise 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 (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Pandey & Rizvi, 2009) hese compounds may be classified into different groups as a function of number of phenol rings that they contain and of the structural elements that bind these rings to one another. The main groups of polyphenols are: flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and lignans. Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Pandey & Rizvi, 2009).

II.1.1 Classification of phenolic compounds

Phenolic compounds are subdivided into groups (Figure 4) by the number of phenolic rings and of the structural elements that link these rings (Han, Shen, & Lou, 2007).

II.1.1.1 Phenolic acids

Phenolic acids are non-flavonoid polyphenolic compounds which can be further divided into two main types, benzoic acid and cinnamic acid derivatives based on C1–C6 and C3–C6 backbones (Figure 3). While fruits and vegetables contain many free phenolic acids, in grains and seeds—particularly in the bran or hull—phenolic acids are often in the bound form.

These phenolic acids can only be freed or hydrolyzed upon acid or alkaline hydrolysis, or by enzymes (Tsao, 2010).

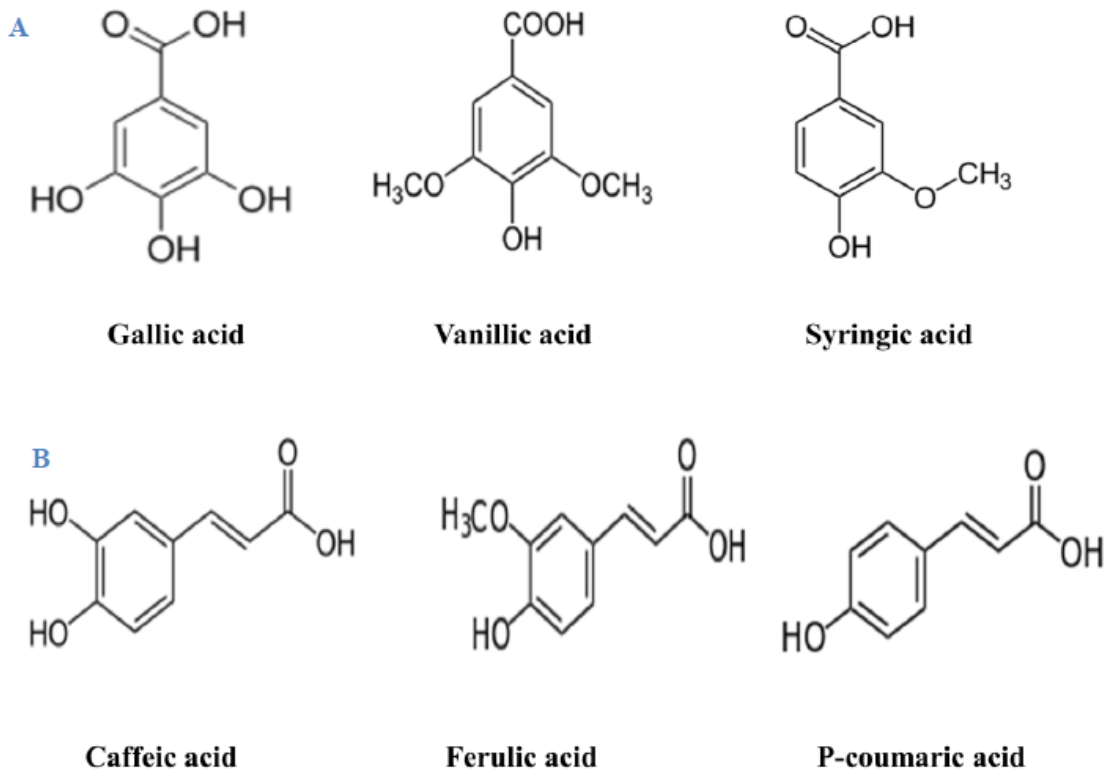

Figure .3. Typical phenolic acids in food: (A) Benzoic acids, (B) Cinnamic acids.

II.1.1.2 Flavonoids

Flavonoids are the most abundant polyphenols in our diets. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), which are labeled as A, B and C. Flavonoids are themselves divided into six subgroups **(Figure 4)**: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation or glycosylation

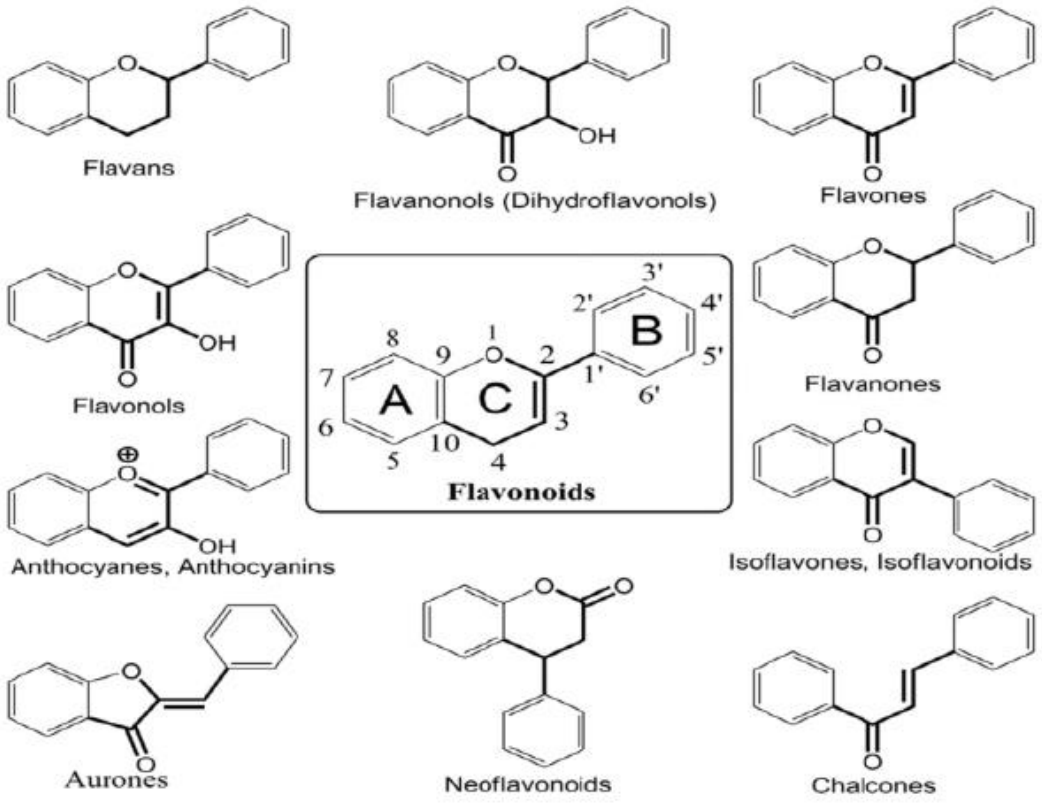

Figure 4: Classification of flavonoids based on the structure of the C2-C4 carbon chain.

II.1.1.3 Tannins

Tannins are relatively high-molecular-weight compounds, and they can be subdivided into hydrolyzable and condensed tannins (Waksmundzka-Hajnos & Sherma, 2010). Hydrolysable tannins are derivatives of gallic acid that is esterified to a core polyol, and the gallyol groups may be further esterified or oxidatively cross-linked to yield more complex hydrolysable tannins. The simplest form is the gallotannins. They are simple polygalloyl esters of glucose. The prototypical gallotannin is pentagalloyl glucose (Figure 5).Condensed tannins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bond (Figure 6). They are also referred to as proanthocyanidins because they are decomposed to anthocyanidins through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions (Dai & Mumper, 2010).

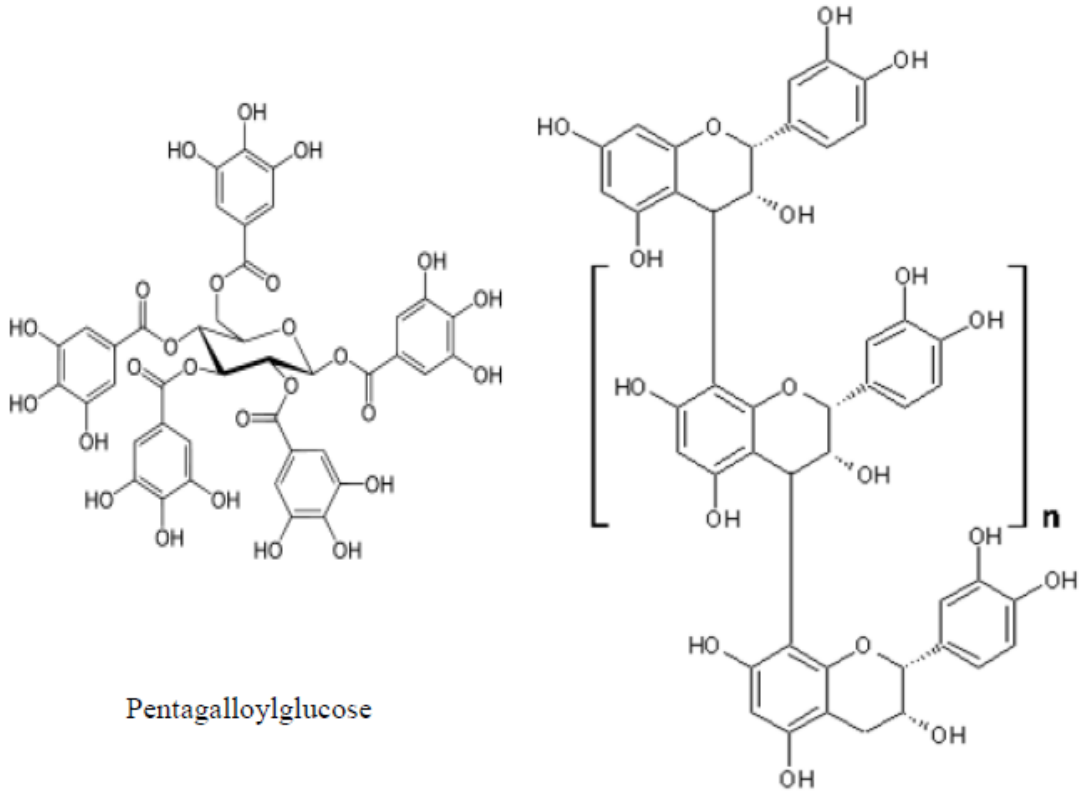

Figure 5: Chemical structure of tannins.

II.I.2 Typical phenolic compounds in artichoke

In the study carried out by Negro, Montesano, Grieco, Crupi, Sarli, De Lisi, et al. (2012) and after HPLC separation, 14 compounds were identified, belonging to hydroxycinnamate and flavone groups (Table III Figure.6.). Identification of some acid compounds (3 to 6 and 11) and flavonoids (8 and 12, Figure 1, Table 1) was based on the comparison of their UV spectra and retention times with those of reference substances.

However, HPLC coupled to mass spectrometry was also used to confirm peak assignment and further characterization of individual substances for all compounds. Mass spectrometric behavior (Table 1) was identified on the basis of pseudomolecular ion or fragmentation patterns in the MS and by comparing the elution order with that reported in the literature; in accordance with the methods of S`anchez-Rabaneda and others (2003), Sch¨utz and others (2004), and

Romani and others (2006), all the qualitative profiles were identified in each artichoke head part and leaf type (Negro, et al., 2012).

Table III–UV Spectra and characteristic ions of caffeoylquinic acids and flavones in artichoke (Negro, et al., 2012)

Nr.	Compound	Retention time (min)	HPLC-DAD λmax (nm)	[M-H]−a m/z	MS/MS[b] m/z	Comparison with standard
1	1-O-caffeoylquinic acid	6.21	233, 305sh, 328	353	191	no
2	3-O-caffeoylquinic acid	8.65	241, 303sh, 325	353	191	no
3	5-O-caffeoylquinic acid	10.48	241, 305sh, 326	353	191	yes
4	4-O-caffeoylquinic acid	11.51	236, 303sh, 326	353	191	no
5	Caffeic acid	12.30	237, 302sh, 323	179	135	yes
6	1,3-di-O-caffeoylquinic acid	13.59	242, 307sh, 322	515	353	yes
7	Luteolin 7-O-rutinoside	25.01	256, 266sh, 350	593	285	no
8	Luteolin 7-O-glucoside	26.13	253, 266sh, 347	447	285	yes
9	Luteolin 7-O-glucuronide	26.88	254, 268sh, 343	461	285	no
10	Apigenin 7-O-rutinoside	29.32	249, 304sh, 328	577	269	no
11	1,5-di-O-Caffeoylquinic acid	29.97	243, 303sh, 329	515	353	yes
12	Apigenin 7-O-glucoside	30.74	229, 266, 339	431	269	yes
13	Apigenin 7-O-glucuronide	31.76	267, 335	445	269	no
14	Luteolin	43.74	254, 266sh, 347	285		no

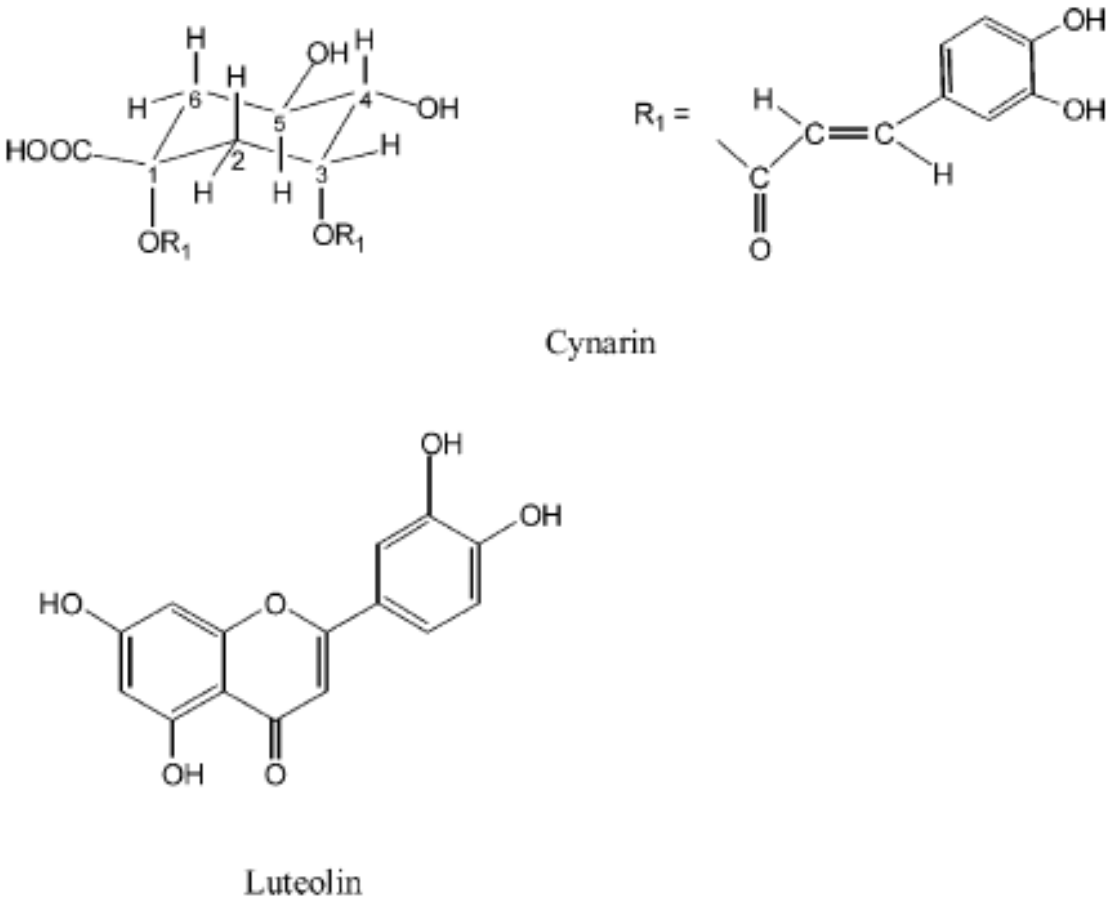

Figure. 6. Typical compounds cynarin and luteolin in artichoke lives (Mulinacci, Prucher, Peruzzi, Romani, Pinelli, Giaccherini, et al., 2004)

II.2 Mechanism of phenolic compounds action in the antioxidant capacity

Over the past years, numerous joint experimental and theoretical studies rationalized the structure-antioxidant properties for large series of polyphenols. Different physico-chemical parameters (descriptors) have been identified to correlate with the antioxidant capacity (Trouillas, Marsal, Siri, Lazzaroni, & Duroux, 2006). It has been clearly proved that the B-ring

is the most important site for H-transfer and consequently for the antioxidant capacity. In contrast, the A-ring seems to be less important. The 2,3-double bond also contributes to the

antioxidant activity, as it ensures p-electron delocalization between the B- and C-rings, which contributes to the stabilization of RO● radical , after H-abstraction (Vaya, Mahmood, Goldblum, Aviram, Volkova, Shaalan, et al., 2003).

The major mechanism of free-radical scavenging has been shown to be H-atom transfer from the active OH group(s) of the phenolic antioxidant (ArO-**H**) to the free radical (R**)** (Di Meo et al., 2013**)**, following: **ArO-H + R• → ArO• + R-H**

This process was drive by different possible mechanisms (Benayahoum, Amira-Guebailia, & Houache, 2014; Ponomarenko, Trouillas, Martin, Dizhbite, Krasilnikova, & Telysheva, 2014).

(i) HAT (H-atom transfer) and PCET (proton-coupled electron transfer):

This is the direct HAT, which is purely governed by the homolytic dissociation of the O–H bond of the active OH groups (ArOH ArO● + H●). PCET is distinguished from the pure HAT as it involves several molecular orbitals. This mechanism occurs in an H-bonding pre-reaction complex in which the proton transfer occurs along the H-bond to one of the lone pair of the O-atom of the free radical. This transfer is coupled to the electron transfer that occurs from a lone pair of the antioxidant to the SOMO (singly occupied molecular orbital) of the free radical.

(ii) ET–PT (electron transfer–proton transfer):

Depending on the oxidative system reacting with ArO-**H**, this mechanism could also occur as a secondary mechanism. The first step of this reaction (ArO-**H** + R• → ArOH+• + R-) leads to the formation of the radical cation ArOH+•, which easily undergoes heterolytic dissociation leading to the same final products than those yielded by PCET (i.e. ArO• + R-**H**).

(iii) SPLET (sequential proton-loss-electron-transfer):

In this mechanism a proton is lost prior to electron transfer from the subsequent anion to the free radical **(i.e. ArO-H → ArO- + H+; ArO- + R• → ArO• + R-; R-+ H+→ RH).**

This mechanism is strongly enhanced under alkaline conditions, which may favor the first step (i.e., proton loss). Again the same final products as with PCET and ET–PT are formed (i.e., ArO●+ R-H).

The antioxidative properties of flavonoids are based not only on their free-radical scavenging ability but also on their chelation of transient metals. It is generally accepted that the two neighboring hydroxyls or neighboring carboxyl and hydroxyl groups of flavonoids are responsible for the metal chelating ability of these compounds. In ring B, the 3' and 4'- hydroxyl groups, which are known as catechol groups, are very active in the chelation of metals. In ring C, the 3-hydroxyl-4-carbonyl and 5-hydroxyl-4-carbonyl groups can also participate in metal chelation. The chelation forces are increased in an alkaline environment (pH 10) due to the deprotonation of hydroxyls.

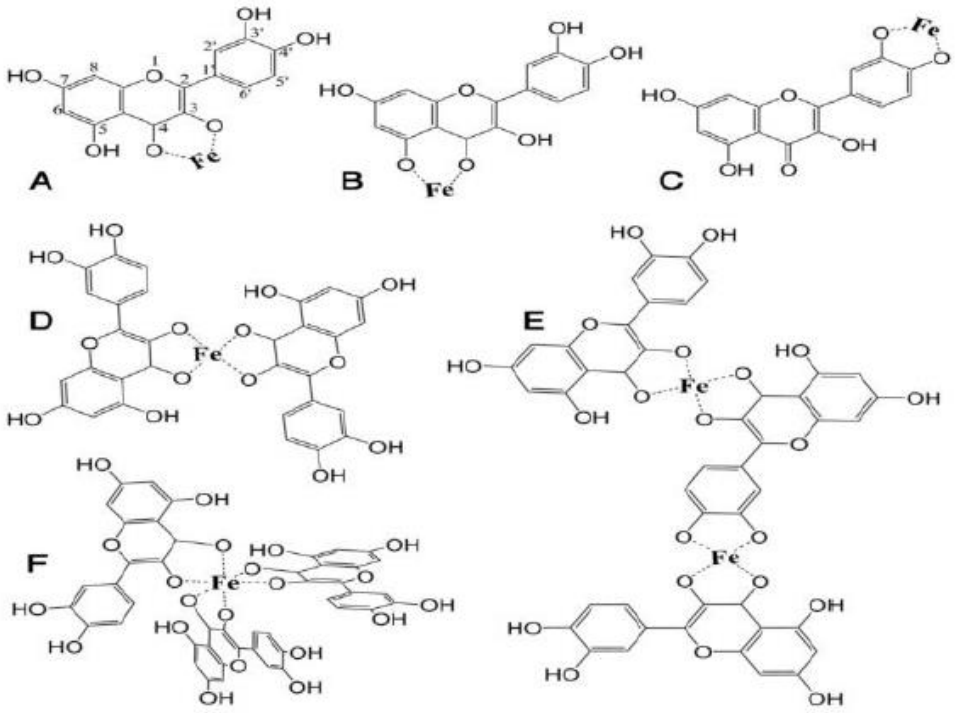

Figure 7: Probable structure of some quercetin–Fe complexes with stoichiometries of 1:1 (a–c), 2:1 (d), 3:2 (e), and 3:1 (f).

II.3 Interaction type between phenolic compounds in complex antioxidants system

Antioxidant efficacy measurement tests must involve antioxidant substances, but some points could lead to ambiguities in measurements and interpreting the results. The first concerns nonlinear synergistic and antagonistic effects that could arise when the substances are mixed. The second concerns the bias that these substances may induce when their efficacy is analyzed.

- **Synergism effect**

Synergism effect is observed when the inhibiting effect of the antioxidant mixtures is higher than the sum of the individual phenolic antioxidants (Kancheva & Kasaikina, 2012)**.** There are mainly four types of synergy. (i) Firstly, the regeneration of highly active antioxidants by less active forms could explain the synergies noted between a-tocopherol and some phenolic compounds (-)-epicatechin, (+)-catechin and quercetin) in a methyl linoleate solution in organic medium. (ii) Secondly, synergies can also be created through the interaction of antioxidants with different mechanisms of action (singlet oxygen quenchers and chain-breaking antioxidants). (iii) In multiphase media, the interaction of antioxidants with different polarities, which are thus distributed in different phases or solvents, could induce synergy, as already discussed with respect to ascorbate-induced regeneration of a tocopherol (Laguerre, Lecomte, & Villeneuve, 2007).

- **Additivism effect**

Additivism effect is observed when the inhibiting effect of the binary mixtures is equal to the sum of the phenolic antioxidants alone.

- **Antagonism effect**

Antagonism is observed when the inhibiting effect of the binary mixtures is lower than the sum of the individual phenolic antioxidants(Kancheva & Kasaikina, 2012);(Peyrat-Maillard, Cuvelier, & Berset, 2003) observed antagonistic effects between a-tocopherol and certain phenolic acids (rosmarinic and caffeic acids) during oxidation of an aqueous dispersion of linoleic acid induced by the azo-initiator 2, 2 0-azobis (2-amidinopropane) dihydrochloride (AAPH). The authors explained this antagonism by the fact that a fraction of these acids (highly active) would regenerate the less active a-tocopherol.

II.4 Antioxidant activity evaluation methods

II.4.1 Chemical antioxidant activity tests

II.4.1.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

- Definition

DPPH is a stable radical in solution and appears purple colour absorbing at 515 nm in methanol (Krishnaiah, Sarbatly, & Nithyanandam, 2011).This assay is based on the principle that DPPH● was reduced by scavenger substance, the purple colour changes to yellow with concomitant decrease in absorbance at 515 nm (Figure 8). The colour change is monitored by spectrophotometrically and utilized for the determination of parameters for antioxidant properties .(Mishra, Ojha, & Chaudhury, 2012). This method is easy to perform, highly reproducible and comparable with other methods such as ABTS, reduction of superoxide anion and inhibition of lipid peroxidation (Villaño, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla, 2007).

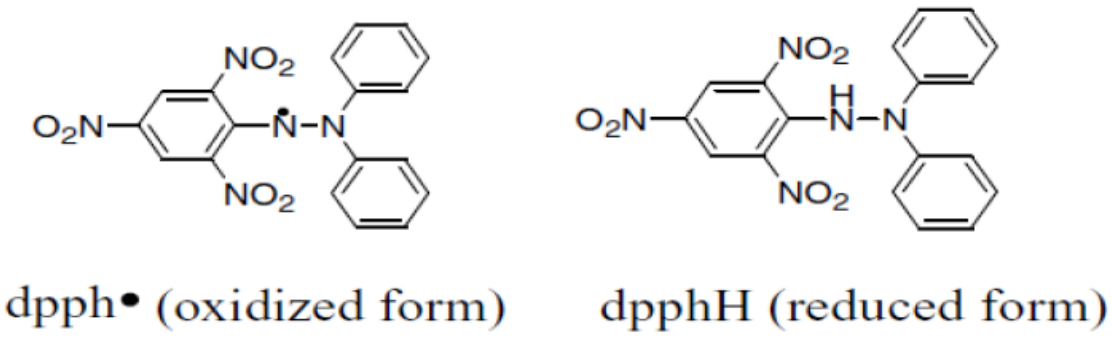

Figure 8: Structures of DPPH radical and reduced form of DPPH

- ***Reaction and Mechanism***

Nitrogen centered radicals such as DPPH• react with phenols (ArOH) via two different mechanisms: (i) a direct abstraction of phenol H-atom (HAT reactions) and (ii) an

electron transfer process from ArOH or its phenoxide anion (ArO−) to DPPH• (ET reactions).The contribution of one or the other pathway depends on the nature of the solvent

and/or the redox potentials of the species involved. Generally in apolar solvents the HAT mechanism is predominant, but in polar solvents, the ET mechanism becomes important (AKELE, HYMETE, & AHMED, 2010; Villaño, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla, 2007)

DPPH• (Violet at 515 nm) + ArOH → DPPH (H) (Colorless) +ArO• (HAT)

DPPH• (Violet at 515nm) + ArOH → DPPH− (Colorless) + [ArOH]•+(ET)

According to the work of Villano et al. (2007) studying the reactivity of phenolic compound with different chemical structure and DPPH radical found a positive relation between the number of OH group and stochiometric number (n) ,which means the number of oxidant molecules reduced by one molecule of antioxidant), as can be seen in the Table IV.

Table IV: Total stoichiometric values *n* of phenolic compounds classified by chemical Structure

Compounds	n	Number of -OH
Phenolic acids		
Gallic acid	5.6 ± 0.2	3
Caffeic acid	2.3 ± 0.1	2
Ferulic acid	1.3 ± 0.0	1
Flavan-3-ols		
(+)-Catechin	4.5 ± 0.3	5
(-)-Epicatechin gallate	7.3 ± 0.6	7
(-)-Epigallocatechin gallate	7.9 ± 0.9	8
Procyanidine B1	7.6 ± 2.3	10
Quercetin	5.2 ± 0.5	5
Myricetin	7.6 ± 0.9	6
Rutin	5.8 ± 0.2	4

Antioxidants of reference		
Ascorbic acid	2.3 ± 0.2	2
Trolox	2.0 ± 0.4	1

II.4.1.2 2, 2’-9-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is a well-known compound used for the colorimetric analysis of various chemical oxidants (Lee & Yoon, 2008).The ABTS radical scavenging method was developed by Rice- Evans and Miller in 1994 and was then modified by Re et al. (1999). This improved method generates a blue/green ABTS•+ chromophore via the reaction of ABTS and potassium persulfate (Figure 9) and is now widely used. Along with the DPPH method, the ABTS radical scavenging method is one of the most extensively used antioxidant assays for plant samples (Krishnaiah, Sarbatly, & Nithyanandam, 2011).

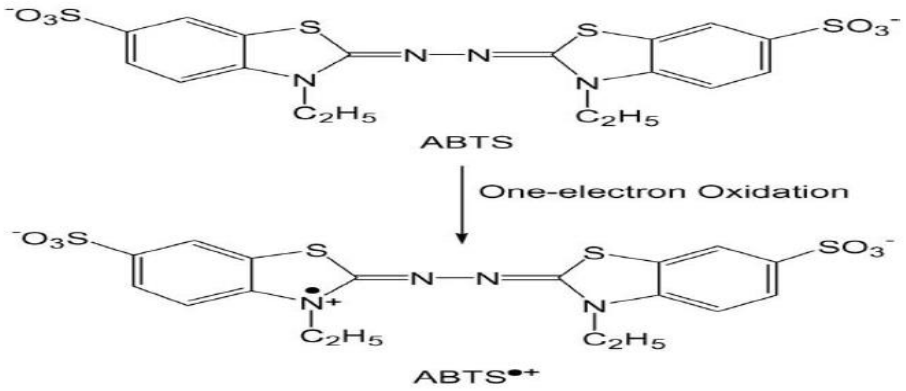

Figure .9. ABTS and its one-electron oxidation product, ABTS•+ (Lee & Yoon, 2008).

- **Reaction and Mechanism**

When the colored ABTS+ is mixed with any substance that can be oxidized, it is reduced to its original colorless ABTS form again; in contrast, the reacted substance is oxidized.

This feature is the basic principle of the methods that use ABTS (Erel, 2004). Though the ABTS assay is generally accepted as a single electron transfert (SET) assay, but ABTS•+ can be

neutralized by SET and HAT mechanisms. The HAT and SET assay reaction schemes are as follows(Craft, Kerrihard, Amarowicz, & Pegg, 2012) :

ABTS• + (Green at 734 nm) + ArOH → ABTS (Colorless) + [ArOH]•+ (SET)

ABTS• + (Green at 734 nm) + ArOH → ABTS (H) (Colorless) +ArO • (HAT)

II.4.1.3 Oxygen radical absorbance capacity (ORAC) assay

The ORAC method, developed initially by (Cao, Sofic, & Prior, 1997) consists of measuring the decrease in the fluorescence of a protein as a result of the loss of its conformation when it suffers oxidative damage caused by a source of peroxyl radicals (ROO) (Figure 10). The method measures the ability of the antioxidants in the sample to protect the protein from oxidative damage. In this assay, fluorescein as the target protein (Zulueta, Esteve, & Frígola, 2009) and the fluorescent loss of this probe is followed over time in the absence and presence of antioxidant (Niki, 2010). 2, 2′-azobis (2-amidino-propane) dihydrochloride (AAPH) is used as the source for the peroxyl radical, which is generated as a result of the spontaneous decomposition of AAPH at 37 ± 1°C (Ou, Chang, Huang, & Prior, 2013). Recently, a novel ORAC assay to assess antioxidant capacity of phenolic compounds in near-gastric conditions (pH 2.0) is presented where only pyrogallol red (PGR) as target molecules showed a behavior compatible with an ORAC assay under acidic conditions (ORAC-PGR) (Atala, Aspée, Speisky, Lissi, & López-Alarcón, 2013). This method was widely used in the evaluation of antioxidant capacity of various samples as fruits and vegetables, dietary supplements, wines, juices, and nutraceuticals, and also the ORAC assay have been used in plasma or serum samples (Karadag, Ozcelik, & Saner, 2009).

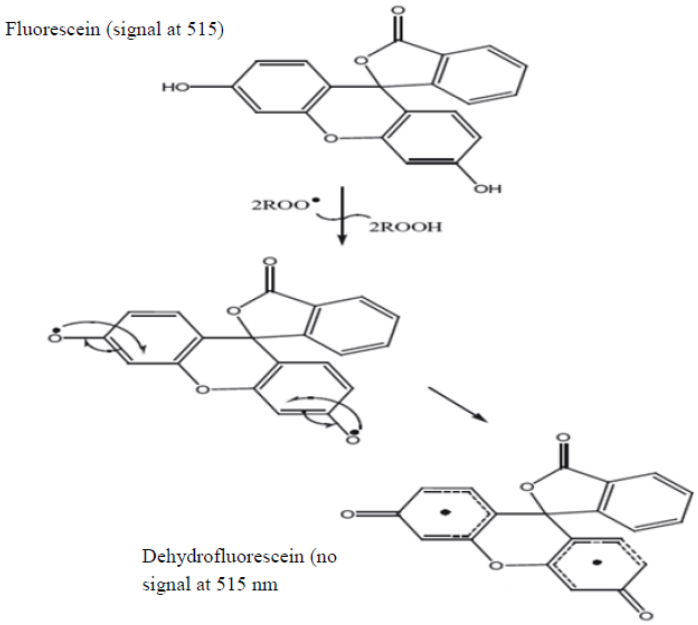

Figure .10. Oxidation of fluorescein to fluoresceinyl radical by ROO radicals from AAPH (Craft, Kerrihard, Amarowicz, & Pegg, 2012).

- ***Reaction and Mechanism***

ORAC measures antioxidant inhibition of peroxyl-radical induced oxidations and reflects classical radical chain breaking antioxidant activity by H-atom transfer (HAT-based method) (Ou, Chang, Huang, & Prior, 2013). A proposed scheme of the major reactions involved in the ORAC assay is listed below, showing the reactions of the peroxyl radicals (ROO) with fluorescein (FH) and antioxidant (AH) (Bisby, Brooke, & Navaratnam, 2008):

AAPH+ 2O2 $2ROO^{\bullet}$ (1)

ROO● + AAPH Product (2)

FH + $ROO^{\bullet}$ $F^{\bullet}$ + ROOH (3)

$F^{\bullet}$+$F^{\bullet}$ Products (4)

AH+ $ROO^{\bullet}$ ROOH + $A^{\bullet}$ (5)

$A^{\bullet}$+$A^{\bullet}$ products (6)

$F^{\bullet}$+AH FH + $A^{\bullet}$ (7)

This scheme describes a simple kinetic competition between FH and AH for reaction with the peroxyl radicals and when the reactivity of the antioxidant toward free radicals is much higher than that of the probe, a clear lag phase is produced (Takashima, Horie, Shichiri, Hagihara, Yoshida, & Niki, 2012) which correspond to the fluorescence stability of the probe

used. This parameter is related to the effectiveness of the antioxidant tested in ORAC assay, i.e higher lag phase means higher antioxidant activity (Bisby, Brooke, & Navaratnam, 2008).

II.5. Roles of phenolic compounds

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

II.5.1. 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 pollinatorsand as plants' chemical defence mechanism against infections caused by

microorganisms and injuries by insects(Hurtado-Fernández, Gómez-Romero, Carrasco-Pancorbo, & Fernández-Gutiérrez, 2010; Soto, Moure, Domínguez, & Parajó, 2011).

II.5.2 **In nutrition and human physiology**

Reactive oxygenic species (ROS) in the form of superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) are natural by-products of human metabolism. However, when present in excess, they can attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with degenerative diseases(Barreira, Ferreira, Oliveira, & Pereira, 2008). 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, Jamei, & Esfahlan, 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 greatinterest in the food industry because

they improve the quality and the nutritional value of foods. When added to foods, antioxidants minimise rancidity, retard the formation of toxic oxidation products, maintain nutritional

quality, and increase shelf life (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002; Proestos & Komaitis, 2008).

II.6. Phenolic compounds from agro-industrial by-products

In recent decades, fruit and vegetable consumption has attracted growing interest because many epidemiological and biochemical studies have consistently demonstrated a clear and significant positive association between intake of these natural food products, consumed regularly as part of the Mediterranean diet, and reduced rates of heart disease, common cancers, and other degenerative diseases, as well as aging. The protection that fruits and vegetables

provide against these maladies has been attributed to the presence of several antioxidants, especially to antioxidative vitamins, including ascorbic acid (vitamin C), α- tocopherol (vitamin E) and ß-carotene (provitamin A). Nevertheless, recent studies seem to indicate that (poly) phenolic substances are the main phytochemicals with antioxidant properties found in higher plants (Garcia-Salas, Morales-Soto, Segura-Carretero, & Fernández-Gutiérrez, 2010).

Amongst fruits, vegetables and different herbs, agricultural and industrial residues are attractive sources of natural antioxidants (Ignat, Volf, & Popa, 2011; Moure, Pazos, Medina, Domínguez, & Parajó, 2007; Turkiewicz, Wojdyło, Tkacz, Nowicka, & Hernández, 2019). 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, Sundram, & Samman, 2006). Phenolic compounds with antioxidant activity have been identified in several agricultural by-products, such as rice hulls , buckwheat hulls and almond hulls (Wojdyło, Oszmiański, & Czemerys, 2007).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylatedhydroxytoluene (BHT) and tertbutylhydroquinone (TBHQ) have been used widely as

Chapter III: Microwave Assisted Extraction (MAE)

III.1 History

The use of microwave energy in sample treatment has attracted growing interest in the past few years (Camel, 2000). Microwave radiation was discovered as a heating method in 1946, with the first commercial domestic microwaves being introduced in 1950 (Mallakpour & Rafiee, 2011). In 1975 Abu-Samra et al. were the first researchers ever to use a microwave domestic oven in the laboratory performing trace analysis of metals from biological samples(Sparr Eskilsson & Björklund, 2000).The first commercial microwave for laboratory utilization was recognized in1978(Mallakpour & Rafiee, 2011).Since microwave irradiation was used in the recovery of the important components from plant materials in 1986,there have been numerous reports on microwave assisted extraction of plant secondary metabolites(Zhang, Yang, & Wang, 2011). Although microwave energy has great potential for rapidly heating materials, microwave ovens have only recently appeared in analytical laboratories.

Nowadays this technology is being applied not only in analytical chemistry but also in organic synthesis, inorganic reactions, preparation of catalysts, and other fields(Nóbrega, Trevizan, Araújo, & Nogueira, 2002).

III.2 Microwaves are Electromagnetic Fields

Microwave is an electromagnetic radiation with a wave length from 0.001 m to 1 m (Figure.11) . (i.e. with a frequency from 3×10^{11} Hz to 3×10^{8} Hz), which can be transmitted as the wave (Zhang, Yang, & Wang, 2011). Generally radar transmissions use the wave lengths between 0.01and0.25m and telecommunications uses the remaining wavelengths. All microwave reactors for chemical synthesis and all domestic microwave ovens operate at 2.45GHzfrequency, which corresponds to a wavelength of 12.25 cm. This is in order to avoid any interference with telecommunications and cellular phone frequencies. The microwave region of the electromagnetic spectrum is shown in Fig.7. As indicated in this figure, this region lies between the infrared and radio frequencies(Motasemi & Ani, 2012)

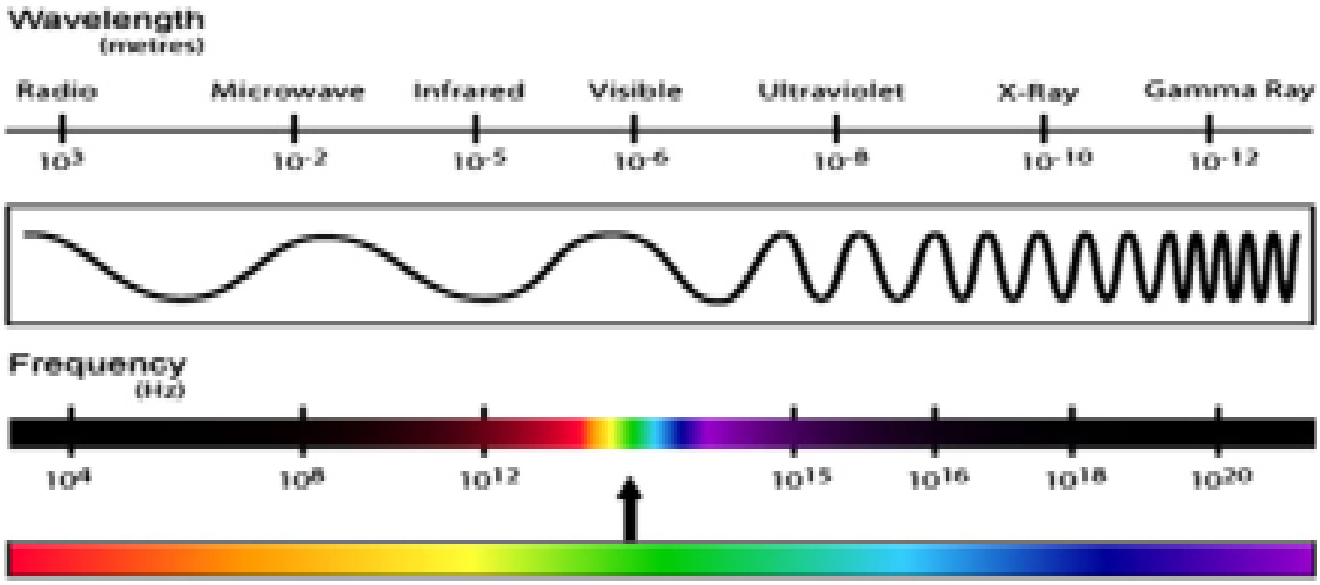

Figure 11: The electromagnetic spectrum.

III.3 Principe

Microwave irradiation is a well-established method of accelerating and enhancing chemical reactions because it delivers the energy directly to the reactant(Motasemi & Ani, 2012).Therefore, heat transfer is more effective than in conventional heating and the reaction can be completed in a much shorter time(Motasemi & Ani, 2012).

When microwave passes through the medium, its energy may be absorbed and converted into thermal energy(Zhang, Yang, & Wang, 2011).Microwave heating is based dielectric heating, the ability of some polar liquids and solids to absorb and convert microwave energy into heat. The orientation of the dipoles changes with the magnitude and the direction of the electric field. Molecules that have a permanent dipole moment are able to align themselves through rotation, completely or at least partly, with the direction of the field. Therefore, energy is lost in the form of heat through molecular friction and dielectric loss. The amount of heat produced by this process is directly related to the capability of the matrix to align itself with the

frequency of the applied electric field. If the dipole does not have enough time to realign, or reorients too rapidly with the applied field, no heating occurs(Mallakpour & Rafiee, 2011).

II1.4. Mechanism of Microwave Heating

Microwave heating is caused by the ability of the materials to absorb microwave energy and convert it into heat. Microwave heating of food materials mainly occurs due to dipolar and ionic mechanisms. The presence of moisture or water causes dielectric heating due to the dipolar nature of water (Lucchesi, 2005). When an oscillating electric field is incident on the water molecules, the permanently polarized dipolar molecules try to realign in the direction of the electric field. Due to the high frequency the electric field, this realignment occurs at a billion (2.4×10^9) times per second and causes internal friction of molecules resulting in the volumetric heating of the material. Microwave heating might also occur due to the oscillatory migration of ions in the food which generates heat in the presence of a high frequency oscillating electric field (Chandrasekaran, Ramanathan, & Basak, 2013). There are many factors which affect microwave heating and its heat distribution and the most important are the dielectric properties and penetration depth.

II1.4.1. Dielectric properties

Microwave can penetrate into certain materials and interacts with the polar components to generate heat. The heating of microwave energy acted directly on the molecules by ionic conduction and dipole rotation (Eskilsson & Björklund, 2000) and thus only selective and targeted materials can be heated based on their dielectric constant. The efficiency of the microwave heating depends on the dissipation factor of the material, tan ı, which measures the ability of the sample to absorb microwave energy and dissipate heat to the surrounding molecules as given by Eq. (1) (Mandal, Mohan, & Hemalatha, 2007)

$$tan\delta = \frac{\varepsilon''}{\varepsilon'} \quad (1)$$

Where ε'' is the dielectric loss which indicates the efficiency of converting microwave energy into heat while $\varepsilon''$is the dielectric constant which measures the ability of the material to absorb microwave energy. The rate of conversion of electrical energy into thermal energy in the material is described by Eq. (2) (M. Chen, Siochi, Ward, & McGrath, 1993)

$$P = K.f\varepsilon' E^2\, tan\delta \quad (2)$$

where P is the microwave power dissipation per unit volume, K is a constant, *f* is the applied frequency, $\varepsilon'$is the material's absolute dielectric constant, E is the electric field strength and tan δ is the dielectric loss tangent.

III.4.2. Dipole rotation

One of the interactions of the electrical field component with the matrix is called the dipolar polarization mechanism. For a substance to generate heat when irradiated with microwaves it must possess a dipole moment, as has a water molecule. A dipole is sensitive to external electric fields and will attempt to align itself with field by rotation (Figure.12.), the applied field provides the energy for this solution. The ability of molecules in a liquid to align with applied electrical field will vary with different frequencies and with a viscosity of the liquid. Under low frequency irradiation, the molecule will rotate in phase with oscillating electric field. The molecule gains some energy by this behaviour, but the overall heating by this full alignment is small (Delazar, Nahar, Hamedeyazdan, & Sarker, 2012).

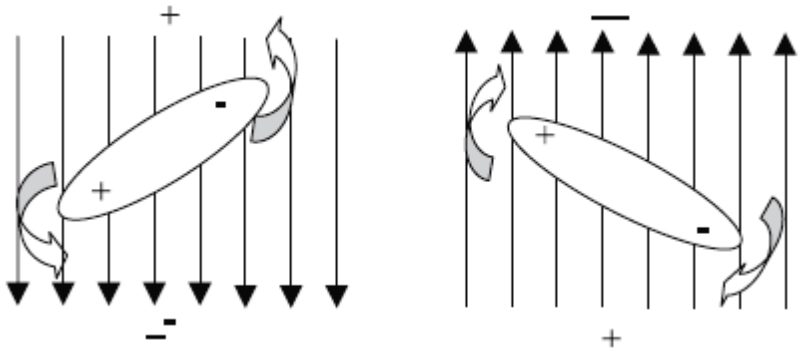

Figure .12. Realignment of a dipole in an electromagnetic field (Al-Harahsheh & Kingman, 2004)

II1.4.3. Ionic conduction

Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied. The resistance of the solution to this flow of ions will result in friction and, thus, heat the solution. As the concentration of ions increases in solution, more collisions occur, causing the solution to heat faster.

The strength of the reaction medium for ion movement induce the heating effect by the phenomenon of friction, and causing a conduction current Jc (Eq. 3.) (Routray & Orsat, 2012).

$\vec{Jc} = \sigma \vec{E}$ .3.

Jc: conduction current; σ : electric conductivity; E : electric field.

Ions are mainly found in vegetable matrices and migrate under the influence of the electric field, which results in the production of heat. This is highly dependent on the mobility

of ions, their concentration in the medium, the ion charge, size, and finally the temperature of the test solution.

Table V: Physical constants and dissipation factors for some solvents commonly used in MAE at frequency of 2.45 GHz (Mandal, Mohan, & Hemalatha, 2007).

Solvents	Dielectric constant (ε′)	Dissipation factor tan δ	Dielectric loss (ε′′)	Boiling temperature °C
Acetone	20.7			56
Acetonitrile	37.5			82
Ethanol	24.3	0.941	22.866	78
Hexane	1.9	0.040	0.038	69
Methanol	32.6	0.659	21.483	65
Water	80.4	0.153	12.30	82
Toluene	2.4	0.04	12.3	100
DMSO	45.0	0.825	37.125	52
Chloroform	4.8	0.091	0.437	
Acetone	20.7	0.941	22.866	

III.5 Factors influencing the performance of MAE

The efficiency of MAE strongly relies on the selection of the operating conditions and the parameters affecting the extraction mechanisms and yield. The factors that may influence the performance of MAE are solvent nature, solvent to feed ratio, extraction time, microwave power, temperature, sample characteristic, effect of stirring,…etc. It is important to understand

the effects and interactions of these factors on the MAE processes (Chan, Yusoff, Ngoh, & Kung, 2011).

III.5.1 Solvent nature and solvent to feed ratio

The selection of suitable solvent in MAE extraction process depends on the solubility of the target analyte, solvent's penetration and interaction with sample matrix and its dielectric constant (Table V). Aqueous solution of certain organic solvent is desired for some extractions as the presence of water would improve the penetration of solvent into sample matrix and thus enhance heating efficiency. Other organic solvents such as ethanol, methanol, and acetone are also found to be effective in extraction. For instance, methanol was used to extract phenolic compounds from grape skins and seeds and higher yield of polyphenols was obtained as compared to extraction using ethanol but the latter extract had stronger antioxidant properties. Solvent toxicity is also evaluated in selecting suitable solvent for MAE, in general ethanol is, by far, the most used solvent and a good microwave absorber which is suitable for extracting many active compounds from plants. It is important to note that the selection of a solvent for MAE can not be deduced from the conventional extraction methods as solvents that work well in conventional techniques might not be a good solvent for MAE. However, a modifier can be added to the solvent to enhance its overall performance. In addition, ethanol or water can be added into poor microwave absorber such as hexane to improve the extraction efficiency as in the case of ginger extraction by MAE. Moreover, acetone can be enhanced by adding portion of methanol in MAE of curcumin from Curcuma longa(Chan, Yusoff, Ngoh, & Kung, 2011).

Once a suitable solvent has been decided upon, its quantity to feed ratio has to be determined as it affects the extraction yield in most cases. An optimum ratio of solvent to solid ratio ensures homogeneous and effective heating. Excessive solvent causes poor microwave heating as the microwave radiation would be absorbed by the solvent and additional power is required. Low ratio of solvent in solid promotes mass transfer barrier as the distribution of active compounds is concentrated in certain regions which limits the movement of the compounds out of cell matrix . Furthermore, the solvent and feed ratio and the vessel size exert an interactive effect in MAE as claimed by (Ruan & Li, 2007) where by a combination of the factors affects the efficiency of extraction especially for closed MAE system .

In plant extraction, high microwave power might cause poor extraction yield due to the degradation of thermal sensible compounds. In general, the extraction yield increases

proportionally with increasing microwave power up to a limit before the increase becomes insignificant or decline . Microwave power provides localized heating in the sample and it acts as a driving force for MAE to destroy the plant matrix so that analyte can diffuse out and

dissolve in the solvent. Increasing the power will generally improve the extraction yield and result in shorter extraction time (Eskilsson & Björklund, 2000).

As power level alone does not give sufficient information regarding the absorbed microwave energy into the extraction system, Alfaro and co-workers introduced a term known as energy density, power per mass for a given unit of time, to investigate the effect of microwave power on MAE. They reported that once the plant matrix is destroyed by microwave radiation, the active compounds will be released. Higher power level does not give any contribution to the investigation of interaction between microwaves and extraction solvent with the sample. Temperature and microwave power are interrelated as high microwave power can bring up the extraction temperature of the system. Increasing the temperature causes the solvent power to increase due to a drop in viscosity and surface tension .High microwave power of MAE beyond the optimum operating power reduces extraction yield as thermo sensible compounds would risk thermal degradation(Alfaro, Bélanger, Padilla, & Paré, 2003).

III.5.2 Extraction time and cycle

Apart from interactive effect on temperature, the influence of the microwave power can be extended to the extraction time. Over exposure to microwave radiation even at low temperature or low operating power was found to decrease the extraction yield due to the loss of chemical structure of the active compounds. In order to avoid the risk of thermal degradation and oxidation, the extraction time of MAE usually varies from few minutes up to half an hour(Cravotto, Boffa, Mantegna, Perego, Avogadro, & Cintas, 2008).

If longer extraction time is required, the risk of thermal degradation can be reduced through extraction cycle. This can be manipulated by feeding fresh solvent to the residue and repeating the extraction step to ensure the completion of the extraction. Chen et al. reported that two extraction cycles of 5 min each are needed to optimize the MAE of triterpenoid saponins from Ganoderma atrum while Yan et al. discovered that 3 extraction cycles at 15 min extraction time are best for extracting astragalosides from R. astragali. The total number of cycles required differs from case to case and it should be justified to save the overall extraction time and the solvent consumption for the extraction(Zheng, Wang, Lan, Shi, Xue, & Liu, 2009).

III.5.3 Plant matrix characteristic

Besides the operating conditions discussed in the above sections, the characteristics of the sample also affects on the performance of MAE. The extraction sample is usually dried, powdered and sieved into fine powder prior to the extraction for optimum extraction yield. Too

small particle size would cause difficulty in separating the extract from the residue and additional clean up steps may have to be employed (Pan, Niu, & Liu, 2001). Moreover, fine

sample treated by solvent for 90 min prior to extraction can enhance the heating efficiency of MAE, promote diffusion and improve mass transfer of active compounds to the solvent .However in some of the reported cases, extended pretreatment time did not improve extraction yield as the active ingredients may have leached out from the sample matrix before extraction .The dried sample matrix pretreated with water helps localized heating of microwave (Pan, Niu, & Liu, 2003).

As the extraction proceeds, the moisture in the sample matrix is heated up, evaporated and generated internal pressure within the cell which ruptures the cells to release the active compounds and hence the extraction yield can be improved (L. Wang & Weller, 2006)

From the discussion presented it is clear that particle size, moisture content and solvent pretreatment have considerable effects on the sample matrix for efficient extraction.

III.5.4 Effect of stirring

By introducing stirring in MAE, the negative effect of low solvent to feed ratio on extraction yield can be reduced. Moreover, the mass transfer barrier created by the concentrated active compounds in a localized region due to insufficient solvent can also be minimized resulting in better extraction yield. In other words, agitation accelerates the extraction speed by accelerating desorption and dissolution of active compounds bound to the sample matrix (Ruan & Li, 2007). However, the significance of this parameter is rarely explored and only a few comments and findings have been reported.

Once the influencing parameters are well understood, MAE can be performed under optimum conditions to achieve the best yield as employed by various research groups. This will be presented in the following section(Ruan & Li, 2007).

III.6. Optimum operating conditions

The determination of optimum MAE operating conditions is usually carried out through statistical optimization studies. The optimum operating conditions of MAE based on various studies reported that the most widely used solvent is ethanol with concentration ranging from

40% to 100%, Water is another common extraction solvent (Chan, Yusoff, Ngoh, & Kung, 2011).

The optimum extraction time can be as short as 1 min for extraction of active compound up to an hour for essential oil extraction. In some extraction studies, several extraction cycles are required to optimize the extraction yield. The first mode of operation emphasizes on the power level of microwave radiation where the extraction is carried out for a fixed microwave power through pre-determined extraction time(Yan, Liu, Fu, Zu, Chen, & Luo, 2010). The power of microwave employed usually ranged from 100 to 500 W to provide the impact energy to rupture the cell wall in the extraction. The second mode of operation is more focused on the extraction temperature rather than microwave power. The extraction temperature is set at the desired set point such as 50–100 ◦C by regulating microwave power. This mode of operation is suitable for thermo sensitive compounds.

The third mode of extraction operation is to provide enough impact energy to rupture the cell wall for the extraction as well as to reduce the risk of thermal degradation during the extraction process. This can be done by two alternatives depending on the microwave system employed. The first alternative provides continuous, desired power of microwave radiation to the extraction system until it reaches the set point of the extraction temperature, where the power is regulated to maintain the temperature. The second alternative associates pulse microwave radiation at fixed power to the extraction system(Y. Wang, You, Yu, Qu, Zhang, Ding, et al., 2008).

III.7. Instrumentation

The rapid development in MAE processes has prompted various suppliers to provide improved microwave systems and related instruments for the extraction process. Most of the microwave extractors available are laboratory-built systems based on domestic microwave oven (Chan, Yusoff, Ngoh, & Kung, 2011).

Microwave furnaces consist of three major components; the source, the transmission lines, and the applicator. The microwave source generates the electromagnetic radiation,and the transmission lines deliver the electromagnetic energy from the source to the applicatorin which is the sample to treat(Cendres, 2010)Moreover, many elements can be added and adapted according to the needs for the experiment (Cendres, 2010).

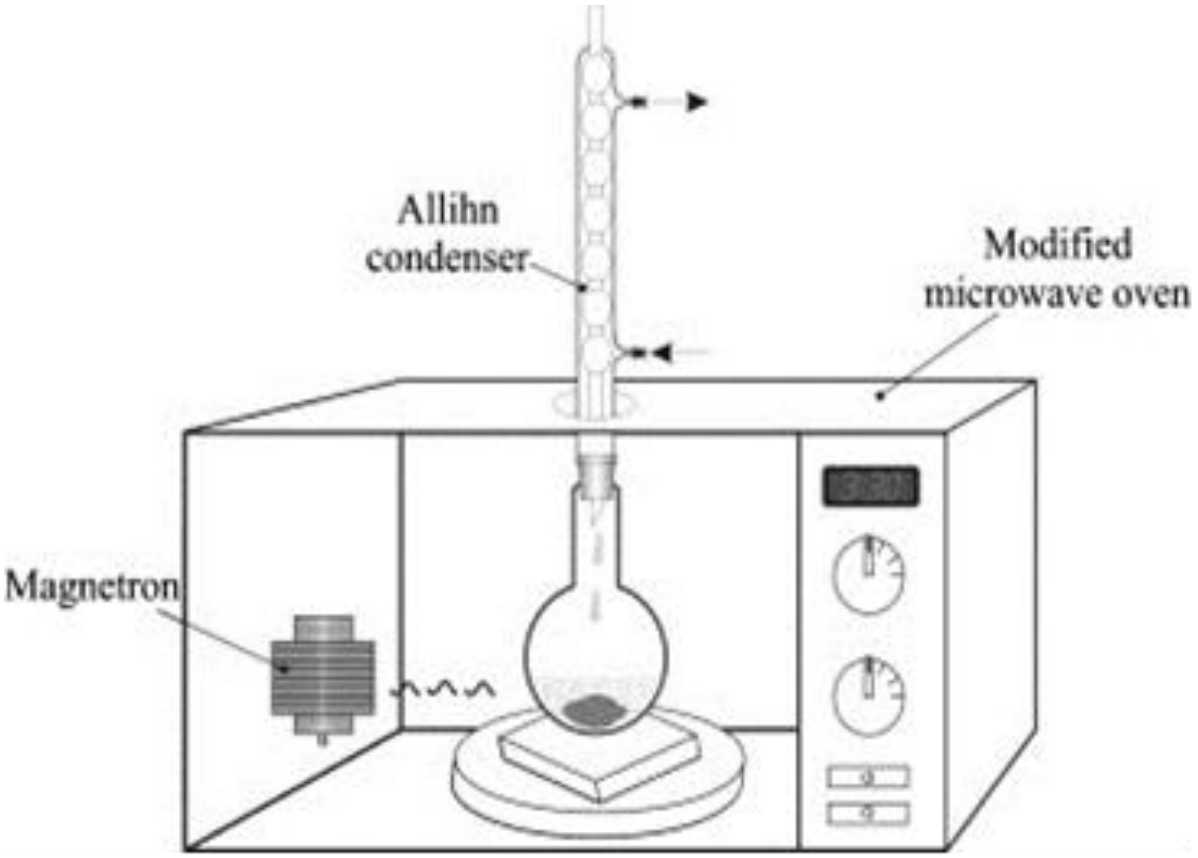

Figure 13: Microwave equipment

III. 8. Advantages of microwave assisted extraction

As detailed above, microwave assisted extraction is suitable for the recovery of a vast array of compounds, and is recognized as a versatile and efficient extraction technique of secondary metabolites from plants. Compared with classical reflux extraction and Soxhlet extraction, microwave assisted extraction generally shows evident advantages with shorter extraction time, higher extraction yield, higher selectivity and better quality of the target extracts. Microwave assisted extraction is relatively cost-effective when compared to accelerated solvent extraction(Zhang, Yang, & Wang, 2011).

The MAE system is considered a promising technique for plant extraction because of its use of different physical and chemical phenomena compared to those in conventional extractions (Veggi, Martinez, & Meireles, 2013).

III.9. Comparison between microwave-assisted extraction (MAE) and other extraction techniques

Many reports (Dean & Xiong, 2000; Eskilsson & Björklund, 2000) on the application and performances of microwave-assisted extraction suggested that MAE is a good and reliable method in sample extraction. In the extraction of active compounds from plant, MAE was reported to be more efficient compared to other conventional extraction methods such as Soxhlet extraction (Soxhlet), heat reflux extraction (HRE), ultrasonicassisted extraction (UAE), and maceration (ME). A comparison between MAE and conventional techniques at their respective optimized conditions that the extraction yield of MAE is higher and required shorter extraction time when compared with other extraction techniques (Y. Chen, Xie, & Gong, 2007). Attributed to its localized heating mechanism, MAE can be completed in just a few minutes as in the extraction whereas it normally takes a few hours for conventional extraction. The advantage of MAE is further supported by the MAE of coumarin and o-coumaric acid from Melilotus officinalis (L.) Pallas (Martino, Ramaiola, Urbano, Bracco, & Collina, 2006), in which 10 min of MAE is sufficient as compared to 60 min by ultrasonicassisted extraction (UAE). Reduction in extraction time from several hours to 3 min was also observed when applying MAE instead of hot solvent extraction (HSE) in the extraction of antioxidant from Folium eriobotryae. Other than that, the ability of MAE in reducing sample preparation time and solvent consumption were also reported in the extraction of volatile organic acids from tobacco leaves (Zhu, Su, Cai, & Yang, 2006).

The distinct advantages of MAE have turned it into a reliable extraction method with high stability and reproducibility suitable to be used in analytical chemistry where precision and

repeatability of analytical result are valued most. In the investigation on the extraction of anthocyanins from grape skins, MAE has successfully quantified the limit that failed to be quantified by the conventional techniques (Liazid, Guerrero, Cantos, Palma, & Barroso, 2011). The high efficiency of MAE is owing to the uniqueness of microwave heating and its interaction with the extraction system enhancing mass transfer. To verify this fact, scanning electron

microscopy (SEM) was employed by several groups to study the mechanism of MAE (Gao, Huang, RoyChowdhury, & Liu, 2007). The results confirmed that microwave radiation has destructive effects on extraction sample matrix and the rapid extraction occurred when the active compounds elute and dissolve in solvent once the cell is ruptured. Most importantly, the therapeutic values of the extracted active compounds can be preserved (Y.-C. Yang, Li, Zu, Fu, Luo, Wu, et al., 2010).

From the economic aspect, MAE is feasible as it requires moderate cost of equipment setup and is much cheaper as compared to non-conventional extraction methods .MAE has low risks and no major safety issues as most extractions are generally carried out under atmospheric condition.

Chapter IV: *General Information on Sunflower oil*

IV. Sunflower oil: composition and health benefits

IV.1. Minor components with a nutritional interest

IV.1.1 Tocopherols

Tocopherols, also known as Vitamin E, are well known antioxidants molecules naturally found in vegetal oils. There are four forms α-, β-, γ- and δ-tocophérol. γ-tocopherol ha showed the highest *in vitro* antioxidant activity followed by δ-tocopherol(Seppanen, Song, & Csallany, 2010). Whereas α-tocopherol presents the highest *in vivo* activity (Traber and Atkinson, 2007) and it exists daily recommended intakes of up to 16 mg/d(Ghazaryan, 2018). Sunflower oil contains a considerable amount of tocopherols but is the oil having the highest amount in α- tocopherol .This content can vary within genotypes and environmental conditions during cultivation(Ayerdi Gotor, Berger, Labalette, Centis, Daydé, & Décriaud-Calmon, 2015). The tocopherols composition could also be modified (Velasco *et al.*, 2004) but is not linked with the composition of fatty acids (Ayerdi-Gotor, Berger, Labalette, Centis, Daydé, & Calmon, 2014).

IV.1.2.Phytosterols

Phytosterols, also known as plant sterols, are a family of compounds which have been studied largely because of their property to reduce the level of cholesterol in blood, but also because of the reduction of the incidence of some cancers(Kritchevsky, 2002). The American National Cholesterol Education Program (Expert panel on detection evaluation and treatment of high blood cholesterol in adults 2001) recommended a daily intake of 2 g of phytosterol to reduce the low density lipoproteins (LDL cholesterol) in blood. The development of food with added phytosterol has led to regulations on the labeling of these products to avoid an excessive consumption (Hung & Verbeke, 2019)and a maximum intake of 3 g/day it has been suggested . Sunflower oil has a high content in phytosterols being β-sitosterol the main sterol. Phytosterol content is mainly affected by environmental conditions during plant growth and genetics but there is no effect of the modification of the fatty acids profile(Ayerdi-Gotor, Berger, Labalette, Centis, Daydé, & Calmon, 2014).

IV.I.3 Others terpenoids

Together with the phytosterols, there are two other terpenoids in sunflower oil, the squalene also a triterpenoid, as the sterols, and the family of carotenoids that are tetraterpenoids. (Rao, Newmark, & Reddy, 1998) concluded that the squalene reduces the risk of colon cancer and the serum cholesterol level. Few studies have focused in the variability of squalene in sunflower oil, (Merah, Langlade, Alignan, Roche, Pouilly, Lippi, et al., 2012) reported a variation from 10 to 202 mg/kg on a collection of inbred lines. Otherwise, sunflower oil it is not particularly rich in carotenoids with only 1–1.5 ppm of carotenoids.

IV.I.4. Phenolic acids

Phenolic acids have largely been studied because of their antioxidants and neuroprotectives properties (Stevenson & Hurst, 2007).Sunflower oil presents two major polyphenols, namely, vanilic acid with 6.9 µg/100 g oil and caffeic acid with 4.9 µg/100 g oil. Moreover, there are also small amounts (each one around 1.5 µg/100 g) of p-hydroxybenzoic, pcoumaric, ferulic and sinapic acid (Siger, NOGALA-KALUCKA, & LAMPART-SZCZAPA, 2008).

IV.I.5. Coenzymes Q9 and Q10

Coenzymes are isoprenoid chains with 6 to 10 isoprenoid units (number indicated after the Q letter) attached to substituted benzoquinone moiety(Pravst, Žmitek, & Žmitek, 2010). Few studies have evaluated the content on coenzyme Q9 (CoQ9) and CoQ10 in oils. (Rodríguez-Acuña, Brenne, & Lacoste, 2008) developed a new method by HPLC MS/MS. They found that sunflower oil had mainly CoQ9 with 101.3 mg/kg and only 8.7 mg/kg of CoQ10 in refined oil whereas other studies showed higher amounts of CoQ10 up to 15 mg/kg in crude oil. These coenzymes are interesting because they have antioxidant and anti-inflammatory activities(Y.-K. Yang, Wang, Chen, Yao, Yang, Gao, et al., 2015) .

IV.2 Other constituents with undesirable functions

IV.2.1 Phospholipids

Crude sunflower oil presents high content of phospholipids, which are the major constituents of the biological membranes. The main families of phospholipids found in sunflower oil are phosphatidylcolines, phosphatidylethanolamides, phosphatidylinositols and phosphatidic acids(Gunstone, 2011). Sunflowers has mainly hydratable phospholips but also

non hydratable phospholipids, which content could vary in function of the activity of the D phospholipase who is able to convert hydratable onto non hydratable phospholipids in presence of water(Haraldsson, 1983). These molecules have unfavorable effects during the refining process as they can saturate bleaching earths or induce browning during deodorization. This affects the flavor, odor and appearance of the oil, they have, therefore, to be removed during the refining process at the degumming stage. However, polyphenols have a role during oil storage increasing the oxidation stabilityand could have beneficial effects on human health (Küllenberg, Taylor, Schneider, & Massing, 2012).

IV.2.2 Free fatty acids

The presence of free fatty acids (FFA) in oils may promote oxidation (Frega *et al.*, 1999). The presence of high levels of free fatty acids, or free acidity, is due to wet harvest conditions which promote the action of lipases generating these molecules, as well as moist grains during storage (Beratlief & Iliescu, 1997). In sunflower oil FFA varied from 1.19 to 1.35% (w/w) in regular sunflower oil (Kreps, Vrbiková, & Schmidt, 2014). FFA are neutralized during the refining process in order to reduce their undesirable effects as undesirable flavor.

IV.2.3 Pigments

The two most common pigments present in vegetable oils are carotenoids and chlorophylls. Few studies have evaluated the content of those two families of molecules in sunflower oils as they are present in small quantities and they are eliminated during the refining process (at the bleaching step).(Topkafa, Ayyildiz, Arslan, Kucukkolbasi, Durmaz, Sen, et al., 2013) found that chlorophyll varied from 403 to 1021 ppb in four crude sunflower oils and β-carotene varied from 1692 to 2803 ppm. The refining process reduces the chlorophylls content up to 96% and 80% the β-carotene content (Kreps, Vrbiková, & Schmidt, 2014).

IV.3 Lipid oxidation in foods

Oxidation plays a significant role in the development of rancid flavors, which negatively modify the organoleptic characteristics, and in the formation of oxidized products that may cause a health hazard. Seeds and drupes from which edible oils are extracted contain triterpenic compounds, which are partially extracted in the oil as a part of the unsaponifiable fraction. Several studies have shown that these compounds possess healthy properties, and they have

also been used as a purity parameter to detect olive oil frauds with pomace olive oil however, some papers have reported that high amounts of triterpenes deteriorate organoleptic oil quality (Orozco, Priego-Capote, & Luque de Castro, 2011)

Lipid oxidation can occur via three primary mechanisms: autoxidation, photosensitized oxidation and enzyme catalyzed oxidation. The process of autoxidation is of the most importance when it comes to food products. Autoxidation is a free-radical mediated chain reaction whereby unsaturated fatty acids are attacked by molecular oxygen to form free radicals and a host of other oxidation products that adversely affect taste, texture, safety and nutritional quality of foods (Ballard, 2008).

Autoxidation occurs in three stages: initiation (formation of free radicals), propagation (freeradical chain reaction) and termination (formation of nonradical species).

- ***Initiation***

Initiation is marked by the formation of free radicals via a hydrogen atom abstraction by an oxidizing agent. Potential oxidizing agents include transition metals, singlet oxygen and free radicals.

The abstraction of a hydrogen atom from an unsaturated fatty acid by an initiator leads to the generation of a lipid free radical (L•). L• rapidly reacts with molecular oxygen to form the lipid peroxyl radical (LOO•).

- ***Propagation***

The propagation stage involves the rapid acceleration of the chain reaction begun in initiation. During propagation, the peroxyl radical abstracts a hydrogen atom from another unsaturated fatty acid, generating a lipid hydroperoxide (LOOH) and another L•. Hydroperoxides are highly unstable primary products of oxidation, but do not contribute to the undesirable flavors and odors commonly associated with rancid foods. However, because of

their instability, peroxides continue in the chain reaction and are further degraded into secondary reaction products such as aldehydes, ketones, acids and alcohols. It is these secondary products of oxidation that are responsible for off-odor and off-flavor development in oxidized foods (Ballard, 2008).

- ***Termination***

Termination is the stage in which free radicals begin to bind to one another to form more stable, nonradical species, thus completing one cycle of lipid oxidation.

Initiator

Initiation: LH L•

Propagation: L• + O2 LOO•

LOO• + LH LOOH + L•

Termination: LOO• + LOO• →

LOO• + L• → non-radical products

L• + L• →

Figure .14. Mechanism of lipid autoxidation.

V. Basics concept

V.Basics concept

V.1.General information on Response Surface Methodology (RSM).

The term optimization has been commonly used in analytical chemistry as ameans of discovering conditions at which to apply a procedure that produces the best possible response. Among the most relevant multivariate techniques used in analytical optimization is response surface methodology (RSM)(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

When many factors and interactions affect desired production process response, response surface methodology (RSM) is an effective tool for optimizing the process, which was originally described by Box and Wilson (1951) (Sun, Liu, & Kennedy, 2010).

Response surface methodology (RSM) is a useful technique for the investigation of several input variables which influence the performance and quality characteristics of the product or process under investigation. The technique provides mathematical and statistical procedures to study relationships between one or more responses (dependent variables) and a number of factors (independent variables). RSM enables information to be obtained at less cost and in a shorter time(Karazhiyan, Razavi, & Phillips, 2011).

RSM is a collection of statistical and mathematical techniques used for developing, improving and optimizing processes(Şahin & Şamlı, 2013).

V.2 Terminology

Before beginning the discussion on the applications of response surface in the optimization of analytical methods, it is pertinent to introduce and define some key terms.

Experimental design

Experimental design is a specific set of experiments defined bya matrix composed by the different level combinations of the variables studied(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

Coded factor levels

Experimental designs are often written in terms of coded variables (Hibbert, 2012).

In screening designs, the factors are usually examined at two levels (–1, +1). The range between the levels is the broadest interval in which the factor can bevaried for the system under study and is chosen on the basis of the literature information or earlier knowledge(Vera Candioti, De Zan, Cámara, & Goicoechea, 2014).If there are several factors, each one of them with its field of variation Inside the field of a continuous factor all the values are theoretically possible. One can thus choose there two, three or several levels according to needs for the study(Goupy & Creighton, 2006)

Experimental domain

Experimental domain is the experimental field that must be investigated. It is defined by the minimum and maximum limits of the experimental variables studied(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

Responses or dependent variables

Responses or dependent variables are the measured values of theresults from experiments. Typical responses are the analytical signal (absorbance, net emission intensity, and electrical signal), recovery of an analyte, resolution among chromatographic peaks, percentage of residual carbon, and final acidity, among others.

Residual is the difference between the calculated and experimental result for a determinate set of conditions. A good mathematical model fitted to experimental data must present low residuals values(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

Response surface designs

With the experimental results of a response surface design, a polynomial model, describing the relation between a response and the considered factors, is build. Usually a second-order polynomial model is constructed.

Afterwards, the model can be interpreted graphically and/or statistically. Graphically, the model is visualized by drawing 2D contour plots or 3D response surface plots. A 2D contour plot (Fig.15.a) shows the isoresponse lines as a function of the levels of two factors, while a 3D response surface plot (Fig.15.b) represents the response in a third dimension. From such plots, often the best or optimal conditions are derived. However, one should be aware that, in case three or more factors are considered, a plot as in Fig.15. only represents a part (occasionally a very small) of the entire response surface in the examined domain.

The fit of the model to the data can be evaluated statistically applying either. Analysis of Variance (ANOVA), a residual analysis, or an external validation using a test set. One also can determine the significance of the **b** coefficients in the above model and then eliminate the non-significant ones, for instance, sequentially(Dejaegher & Vander Heyden, 2011).

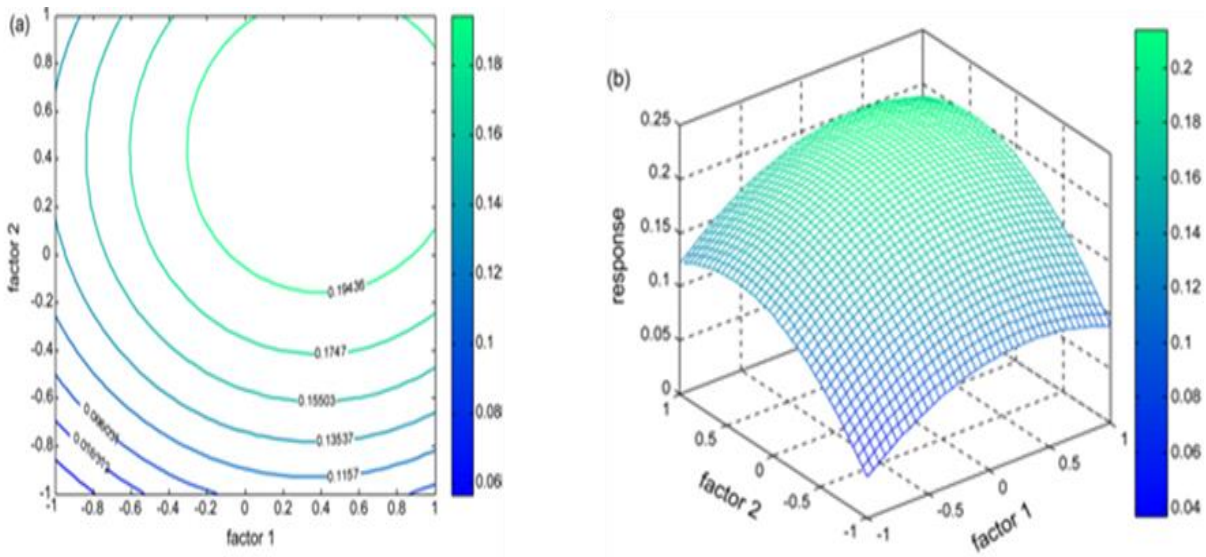

Figure 15: (a) 2D contour plot, and (b) 3Dresponse surface plot

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V.3 Steps for RSM application

Some stages in the application of RSM as an optimization technique are as follows(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008)

V.3.1 Screening of variables

Numerous variables may affect the response of the system studied ,and it is practically impossible to identify and control the(Hibbert, 2012) small contributions from each one. Therefore, it is necessary to select those variables with major effects. Screening designs should be carried out to determine which of the several experimental variables and their interactions present more significant effects(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). They are applied in the context of optimizing separation techniques during screening and in robustness testing, and in the context of optimizing formulations, products, or processes. Most often, two-

level screening designs, such as fractional factorial or Plackett–Burman designs(Dejaegher & Vander Heyden, 2011)are the most widely used in the step of selection of factors because they are economic and efficient.(Vera Candioti, De Zan, Cámara, & Goicoechea, 2014).

V.3.2 Choice of the experimental design

The simplest model which can be used in RSM is based on alinear function. For its application, it is necessary that the responses obtained are well fitted to the following equation:

$$y = \beta_0 \sum_{i=1}^{k} \beta_i X_i + \varepsilon \quad (1)$$

Where *k* is the number of variables, β_0 is the constant term, β_i represents the coefficients of the linear parameters, *xi* represents the variables, and ε is the residual associated to the experiments.

Therefore, the responses should not present any curvature. To evaluate curvature, a second-order model must be used. Two-level factorial designs are used in the estimation of first-order effects, but they fail when additional effects, such as second-order effects,

are significant. So, a central point in two-level factorial designs can be used for evaluating curvature. The next level of the polynomial model should contain additional terms, which describe the interaction between the different experimental variables. This way, amodel for a second-order interaction presents the following terms:

$$y = \sum_{i=1}^{k} \beta_i X_i + \sum_{1 \le i \le j}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

Where βij represents the coefficients of the interaction parameters.

In order to determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (3)$$

Where βii represents the coefficients of the quadratic parameter.

To estimate the parameters in Eq. (3), the experimental design has to assure that all studied variables are carried out at in at least three factor levels. Thus, two modeling, symmetrical response surface designs are available. Among the more known second order symmetrical designs are the three-level factorial design, Box–Behnken design, central composite design, and Doehlert design. These symmetrical designs differ from one another with respect to their selection of experimental points, number of levels for variables, and number of

runs and blocks. These experimental matrices are presented and discussed in Section 4.(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

V.3.3 Mathematical–statistical treatment of data

After acquiring data related to each experimental point of a chosen design, it is necessary to fit a mathematical equation to describe the behavior of the response according to the levels of values studied. In other words, there must be estimates of the *b* parameters of Eqs. (1)–(3). Therefore, in matrix notation, Eqs. (1)–(3) can be represented as

$$y_m X_i = X_{mXn} b_{nX1} + e_{mX1} , \quad (4)$$

where *y* is the response vector, *X* is the matrix of the chosen experimental design, *b* is the vector constituted by the parameters of the model, *e* is the residual, and *m* and *n* represent the numbers of lines and columns from the matrices, respectively.

Eq. (4) is solved by using a statistical approach called the method of least square (MLS). MLS is a multiple regression technique used to fit a mathematical model to a set of experimental daa generating the lowest residual possible. After mathematical transformations

of Eq. (5), a vector *b* containing the parameters can be obtained by the following equation:

$$b_{n\cdot 1} = (X^T_{n\cdot m} X_{m\cdot n})^{-1} (X^T_{n\cdot m} y_{m\cdot i}) \quad (5)$$

Eq. (5) is used in the construction of the response surface that describes the behavior of the response in the experimental domain. The great advantage of Eq. (6) is the low computational cost necessary to determine the *b* coefficients .In the LSM, it is assumed that errors present a random distribution profile with a zero mean and a common unknown variance and that these errors are independent of each other. In this way, the variance estimate to each component of vector *b* is commonly obtained by authentic repetitions of the central point according to Eq. (6):

$$V(b)n\cdot n = (X^T_{n\cdot m} X_{m\cdot n})^{-1} s^2 \quad (6)$$

Thus, extracting the square root for each component of V (b)leads to obtaining the standard errors for the *b* coefficients that compose the equation of the response surface, allowing the evaluation of its significance.(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

V.3.4 Evaluation of the fitted model

The mathematical model found after fitting the function to the data can sometimes not satisfactorily describe the experimental domain studied. The more reliable way to evaluate the quality of the model fitted is by the application of analysis of variance (ANOVA).

The central idea of ANOVA is to compare the variation due to the treatment (change in the combination of variable levels) with the variation due to random errors inherent to the measurements of the generated responses. From this comparison, it is possible to evaluate the significance of the regression used to foresee responses considering the sources of experimental variance(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

Lack of fit test is another way to evaluate the model. it expresses the variation of the data around the fitted model a model will be well fitted to the exprerimental data if it presents a significant regression and a non-significant lack of fit. In other words, the major part of variation observation must be described by the equation of regression, and the remainder of

the variation will certainly be due to the residuals. Most variation related to residuals is due to pure error (random fluctuation of measurements)and not to the lack of fit, which is directly related to the model quality(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

V.3.5 Determination of the optimal

The surfaces generated by linear models can be used to indicate the direction in which the original design must be displaced in order to attain the optimal conditions. However, if the experimental region cannot be displaced due to physical or instrumental reasons, the research must find the best operational condition inside the studied experimental condition by visual inspection(Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

Second Part: Experimental part

Materials and methods

(Part II-A-)

“Section I”

Section I

- ***The optimization and comparison of phenolic compounds extraction from artichoke (Cynara scolymus L.) waste: The determination of the optimal MAE conditions, and the characterization of the stem extracts and by HPLC analysis***
- ***The determination of dietary fiber distribution in different particles size of (Cynara scolymus L.) stem.***

Materials and methods (Part II-A-)

"Section I"

I. Personal work

The effect of different extraction parameters (ethanol concentration, solvent/material ratio, microwave power and irradiation time) on the efficiency and recovery of phenolic compounds from artichoke stem was studied. Box–Behnken design (BBD), which allows the reduction of the number of experiments compared to other statistical designs, BBD is a model of second degree with several variables, it is easy to implement and has the property of sequentiality compared to the factors. We can undertake the study of the first k factors while reserving the option to add new without losing the results of tests already carried out (Goupy & Creighton, 2006). The use of the BBD design is popular in research because it is an economical design and requires only three levels for each factor where the settings are −1, 0, 1.The main characteristics of BBD are:

● The number of experiments (N) required for the development of BBD is defined as $N = 2k(k-1) + C_0$, with k is the number of factors and Pc is the number of central points;

● All levels of factors must be adjusted only at three levels (-1, 0, +1) with regular intervals The experimental points are placed in the middle of the edges of the cube as shown in Figure .16.

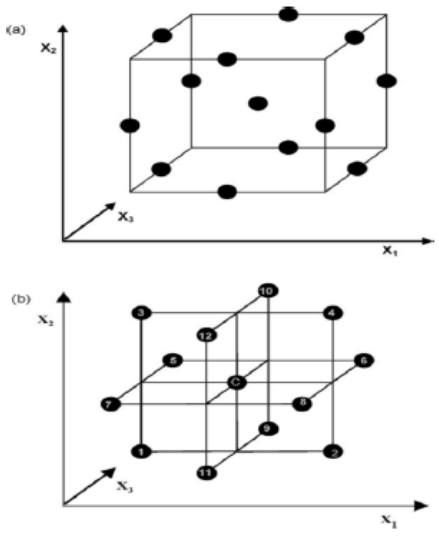

Figure 16 : (a) the cube for BBD and three interlocking 2^2 factorial design (b) (Ferreira, Bruns, Ferreira, Matos, David, Brandao, et al., 2007).

A cube has 12 edges; there will be 12 trials and following the advice of Box and Behnken, adding three points in the center of the study area. BBD plans for three factors contain 15 experimental points. The experimental points are on a sphere, the criterion isovariance by rotation will be respected. Noting, when we place 4 points in the center of the study area, instead of three points, we get a plan that meets the near-orthogonality test The plan BBD for four factors in our case is constructed as like the three factors, the experimental points are located in the middle of the edges of the hypercube which has four dimensions and an adding points to the center give the matrix representation(Goupy, 2005). In this study, four process variables, namely composition of solvent as volume percentage of EtOH (X1), microwave power (X2), irradiation time (X3) and liquid-to-solid ratio (X4) (Table VI), were studied on three levels to investigate their effects on amount of total phenolics in the extract by 2^{nd} order BBD with 24 points, and 3 replications of the center points.

Table .VI. The independent variables and its levels of factors influencing microwave assisted extraction.

Independent variables	Coded Level		
X_1: EtOH (%)	-1	0	+1
X_2: power irradiation (W)	-1	0	+1
X_3: irradiation time (s)	-1	0	+1
X_4: Liquid-to-solid ratio (mL/g)	-1	0	+1

The BBD was combined with RSM to analyse the interaction among the MAE operating factors, such as to maximize the extraction of the phenolic compounds. Additionally, to further confirm the extraction efficiencies, a comparison between MAE and a conventional extraction method (maceration) was done.

The results were treated by RSM, which generates a mathematical model that is adjusted to the obtained experimental responses and this allows the effects of the independent variables to be analysed. The optimum values of these variables were obtained by solving the mathematical equation and the evaluation of the response surface plots. The phenolic content was identified by HPLC-DAD-ESI-MSn analysis then quantified in the enriched sun flower oil using the optimized extract.

I.1 Materials

Fresh artichoks were obtained at a local market and kept refrigerated at +4°C until used. The stems were removed with a knife, washed with distilled water and chopped into pieces of 0.5-1 cm. Stems pieces were dried for about 48 h at room temperature in a ventilated dark room Figure 17.

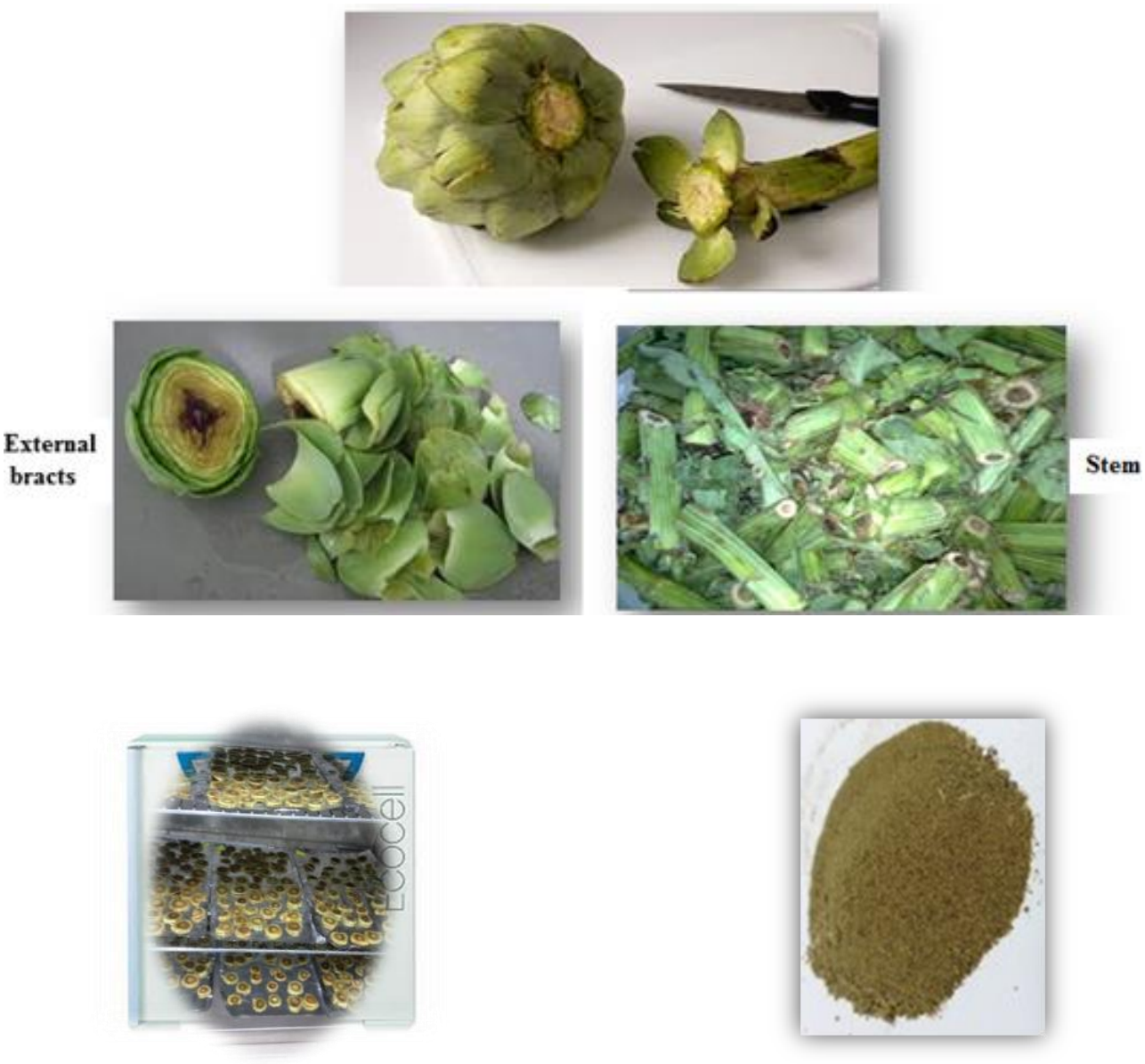

Figure. 17. Samples used during experimental work (Photos from BBBS-Lab).

I.2 Chemicals and solvents

Sodium carbonate (Na_2CO_3), Folin–Ciocalteu phenol reagent were obtained from Prolabo (made in CE). Gallic acid was purchased from Biochem-chemopharma (UK) and 1,1-

diphenyl-2-picryl-hydrazil (DPPH) from Sigma Aldrich (Germany). All solvents used were of analytical grade and purchased from Prolabo (CE).

I.3 Evaluation of moisture content of the sample

Before the extraction experiments, the initial moisture content was determined using thermal drying method. 10 g of sample were placed in an oven at 105 ◦C for 3 h. The moisture content (MC) was calculated by expressing the weight loss upon drying as a fraction of the initial weight of sample used. MC (%) = W0/Wi × 100, where W0 correspond to the loss in weight (g) on drying and Wi correspond to the initial weight of sample (g) (Boulekbache-Makhlouf, et al., 2013).

I.4 Extraction equipment and procedure

I.4.1 Microwave- assisted extraction

Before extraction, the samples were washed with distilled water and cut into washers, and dried for about 48 h at room temperature in a ventilated dark room, then they were ground to obtain a thin powder (≥250 μm diameter).

The extraction of the phenolic compounds from the artichoke by-products was performed in domestic microwave oven system (2.45 GHz, Samsung Model NN-S674MF, Kuala Lumpur, Malaysia). The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter was linearly adjustable from 100 to 1000 W). The oven was modified (Fig. 18) in order to condense the vapours generated during extraction into the sample. For the extraction, one gram of the stem powder was placed in a 250 mL volumetric flask containing the extraction solvent. The suspension was irradiated at regular intervals according to oven operation. Depending on the trial, a different solvent, irradiation time, microwave power and solvent-to-solid ratio were used (Tables VII and VIII). At the end of microwave irradiation, the volumetric flask was allowed to cool to room temperature. After extraction, the extract was recovered by filtration in a Büchner funnel through Whatman No. 1 paper, and collected in a volumetric flask, then stored at (4 °C) until used and analysed for TPC(for the optimized MAE extracts and the sunflower oil).

I.4.2 Experiment design

To reduce the total experimental work and to optimize the MAE approach, the influences of process variables were first examined separately in single-factor experiments. In the MAE experiments when one variable was not studied, it was kept constant. To begin the

experiments different mixtures of solvent/water i.e. , ethanol, methanol and acetone (1:1 v/v; organic solvent: water) were used to identify the adequate solvent for extraction, and then ethanol has been chosen and studied to set the best concentration (20 to 100 %). When testing the influence of both factors (type and concentration of solvent), the constant values of microwave power, irradiation time and solvent / solid ratio were: 500W, 60s and 40:1 mL/g respectively.

After setting the concentration of the solvent (ethanol 60 %), the best ratio was selected by varying the last one from 30:1 to 90:1 mL/g and the constant values of the other factors were the same as those cited above (500W and 60s)

The ratio was fixed at 70:1 mL/g to study the effect of microwave power ranging from 100 to 1000 W, and the other parameters were fixed as follows: ethanol at 60 %, irradiation time at 60 s and 70:1 mL/g for the ratio.

Lastly four irradiation times (1, 2, 3 and 4 min) have been studied, setting the power at 700 W and other factors were the same as those cited above (ethanol 60% , 70:1 mL/g). On the basis of the single-factor experimental results (Tables VI), the major influence factors were selected. Then, an RSM based on a Box–Behnken Design (BBD) was conducted to optimize MAE process (Table VII).

Regression analysis of the data to fit a second-order polynomial equation (quadratic model) was carried out according to the following general equation (Eq. (10)), which was then used to predict the optimum conditions of extraction process.

$$Y = B_0 + \sum_{i=1}^{K} B_i X_i + \sum_{i=1}^{K} B_{ii} X^2 + \sum_{i\rangle j}^{K} B_{ij} X_i X_j + E$$

Eq. (10)

Where *Y* represents the response function (in our case TPC); *B0* is a constant coefficient; *Bi*, *Bii* and *Bij* are the coefficients of the linear, quadratic and cross terms, respectively, and *Xi*,*Xj* represent the coded independent variables. According to the analysis of variance, the regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions, the regression coefficients were used to generate 3-D surface plots from the fitted polynomial equation. The factor levels were coded as −1 (low), 0 (central

point or middle) and 1 (high), respectively. The variables were coded according to the following equation (Eq. (11)):

$$X_i = \frac{x_i - x_0}{\Delta x} \qquad \text{Eq. (11)}$$

Where X_i is the (dimensionless) coded value of the variable x_i and x_0 is the value of X at the center point and Δx is the step change. The analysis of variance was performed for the response variable using the full model, where *P*-values (partitioned into linear and interaction factors) indicated whether the terms were significant or not. To verify the adequacy of the models, additional extraction experiments were carried out at the optimal conditions predicted with the RSM and the obtained experimental data were compared to the values predicted by the regression model. Optimized MAE conditions were then compared to a reference CSE procedure and added to the sunflower oil in order to improve the phenolic content of the sunflower enriched oil and finally compared to the control (unenriched oil).

I.4.3 Statistical analysis

Each extraction trial and all the analyses were carried out in triplicate and all the data in this paper have been reported as means ± SD. Influence of each factor on the TPC yield in the single-factor experiment for the MAE was statistically assessed by ANOVA (analysis of variance) and posthoc Tukey test with 95% confidence level. Data obtained from the BBD trials for the MAE were statistically analysed using ANOVA for the response variable in order to test the model significance and suitability. $p < 0.05$ and $p < 0.01$ were taken as significant and highly significant level, respectively. The JMP (John's MacIntosh Product), Version 7.0, SAS, and Design-Expert (Trial version8.0.7.1) software were used to construct the BBD and to analyse all the results.

Figure.18. MAE apparatus used during extraction of phenolic compounds (Photo from BBBS-Lab).

I.5. Conventional solvent extraction

Phenolic compounds were extracted using a conventional solvent extraction method following the procedure described elsewhere by Zuorro (2014). Briefly, one gram of powder was placed in a conical flask, and an appropriate volume of solvent/water was added. The mixture was kept in a batch mode and magnetically stirred and thermostatically controlled. After the extraction, the sample was recovered by filtration in a Büchner funnel through Whatman N°. 1 paper, and collected in a volumetric flask, then stored at 4 °C until its analysis.

I.6 Total phenolic content (TPC)

TPC of stem extracts was determined according to Georgé, Brat, Alter, and Amiot (2005) method. Briefly, 2.5 mL of diluted Folin-Ciolcateu reagent (diluted ten times 1/10) were added to the extracts. The mixture was incubated for 2 min at room temperature, and 2 mL of saturated sodium carbonate solution (75 g/L) were added. The mixture was incubated for 15 min at 50

°C and finally cooled in a water-ice bath. The specific absorbance at 760 nm was immediately measured by a spectrophotometer (UV-mini 1240, Shimadzu, Japan). The TPC was expressed as milligram gallic acid equivalents per gram of dry weight (mg GAE/ g DW) basis.

I.7 DPPH• radical scavenging assay

The radical scavenging activity of stem extracts and oil was determined by using the DPPH• scavenging assay, according to the method optimized and described by (Neto, Marçal, Queirós, Abreu, Silva, & Cardoso, 2018). Fifty microlitres of the extract/standard were added to 250 µL of the DPPH solution. The mixture was placed in the dark for 30 min and the absorbance was then read at 517 nm.

I.8 UHPLC–DAD–ESI/MS^n analysis

The UHPLC-DAD-ESI/MS^n analysis was performed on an Ultimate 3000 (Dionex) apparatus equipped with an ultimate 3000 diode array detector and coupled to an ion trap mass spectrometer, following the general proceeding as previously described by (Wasli, Jelali, Silva, Ksouri, & Cardoso, 2018). The chromatographic system consisted of a quaternary pump, an autosampler, a degasser, a photodiode-array detector and an automatic thermostatic column compartment. Analysis was run on a HichromNucleosil C_{18} column (250 × 4.6 mm i.d., 5 µm particle diameter, end-capped) and its temperature was maintained at 30 °C. The mobile phase was composed of (A) acetonitrile and (B) 0.1% of formic acid (v/v), both degassed and filtered before use. The solvent gradient started with 90–70% of solvent B over 20 min, from70–40% of solvent B over 10 min, and from 40–0% of solvent B over 5 min.

For the HPLC analysis, each extract (50 mg) was dissolved in 5 mL in apropriate solvent. All samples were filtered through a 0.2 µm Nylon membrane (Whatman). The flow rate was 0.7 mL/min and split out at 200 µL/min to the MS. UV–Vis spectral data for all peaks were acquired in the range 250– 500 nm and chromatographic profiles were recorded at 280 nm. The mass spectrometer used was an Amazon SL (BrukerDaltonics) ion trap MS equipped with an ESI source.

Control and data acquisition were carried out with the Compass Data Analysis data system (BrukerDaltonics, Bremen, Germany). Nitrogen above 99% purity was used and the gas pressure was 520 kPa (75 psi). The instrument was operated in negative-ion mode with electrospray ionization (ESI) needle voltage set at 5.00 kV and an ESI capillary temperature of 200 °C. The full scan covered the mass range from m/z 70 to 700. Collision-induced

dissociation (CID)-/MS and MS^n experiments were simultaneously acquired for precursor ions using helium as the collision gas with collision energy of 10–40 arbitrary units.

Results and discussion

(Part II-A-)

“Section I”

Results and discussion (Part II-A-)

Section I

I. Moisture content of artichoke's stem

The result of the determination of the moisture content of artichoke's stem (is shown in Figure 20. The latter shows that the stem contain a significant amount of water, see 87%.±1.14%. This result is comparable to the content reported by A. Zuorro et al (2016) which is 84.6 ± 1.4 .

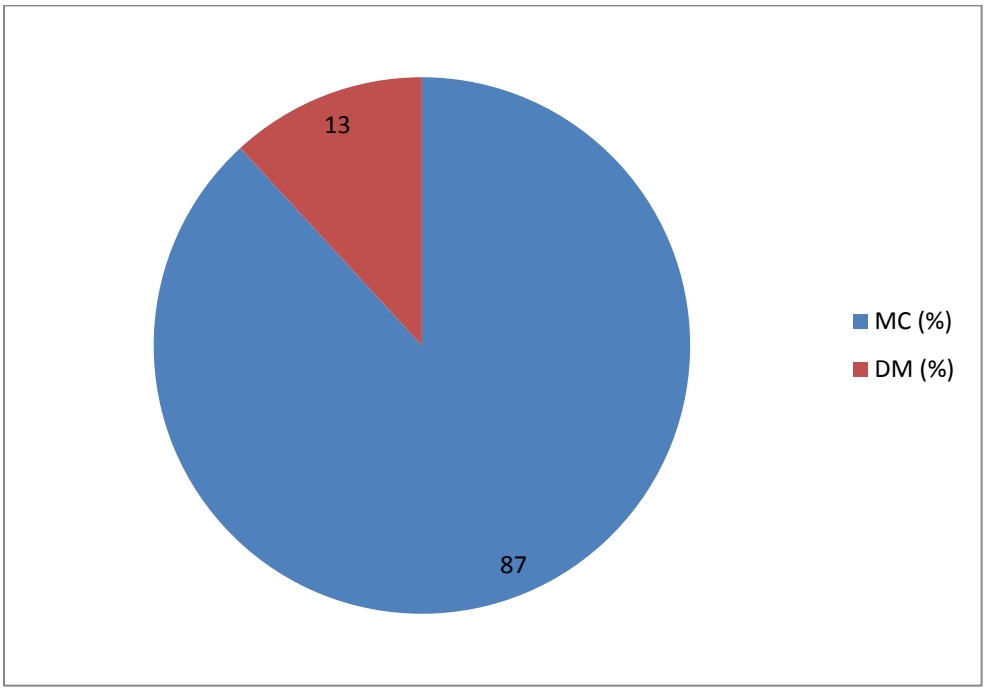

Figure .19. Moisture content of stem.

II.Results of Single-Factor Tests

Preliminary trials were performed to select the experimental range of the MAE factors. The obtained results are summarized in table .VII.

Thus, different mixtures solvent/water i.e., ethanol, methanol and acetone, were tested. The highest outcomes were achieved with the mixtures of ethanol: water (1:1; v/v) (30 ± 2.31 mg

GAE/g DW) and methanol: water (1:1; v/v) (29.72 ± 2.71 mg GAE/ g DW), and the less interesting ones were reached with acetone: water (1:1; v/v). Indeed, ethanol is a safer organic solvent which is used in food and pharmaceutical industries. Even though methanol: water has been described as one of the most efficient solvent for phenolic compounds extraction, ethanol: water (1:1; v/v) was selected as the optimum one for the subsequent RSM optimization studies because the attained yields with both mixtures were not significantly different, and ethanol is a food grade solvent (H. Li, Deng, Wu, Liu, Loewen, & Tsao, 2012). It is also a solvent that can be produced from different bioresources (biosolvent) by fermentation, and can contribute to the sustainability of industrial processes.

Table .VII. Results of single-factor experiments for Microwave-assisted extraction

Factors	**Unit**	**TPC yield (mg GAE/g DW)**	
Solvent	Type	Water	24.51 ± 1.13^{c}
		50 % Acetone	25.63 ± 0.18^{b}
		50 % Methanol	29.72 ± 0.71^{a}
		50 % Ethanol	30.18 ± 1.31^{a}
Ethanol concentration	%	20	26.34 ± 1.30^{c}
		40	29.54 ± 1.05^{ab}
		60	30.63 ± 0.49^{a}
		80	29.75 ± 0.16^{ab}
		100	18.01 ± 0.77^{d}
Solvent-to-solid ratio	m**L/g**	30	20.44 ± 0.62^{d}
		40	28 ± 0.73^{b}
		50	30.14 ± 1.01^{b}
		60	30.42 ± 1.13^{ab}
		70	$31{,}06 \pm 1.12^{a}$
		80	30.8 ± 1.85^{a}
		90	30.06 ± 0.91^{ab}
Microwave power	**W**	100	28.08 ± 0.98^{c}
		300	30.65 ± 1.09^{b}
		500	30.54 ± 0.28^{b}
		700	31.10 ± 0.13^{a}
		900	30.93 ± 0.24^{a}
		1000	28.08 ± 0.98^{c}
Irradiation time	**min**	1	28.95 ± 0.31^{c}
		2	29.51 ± 0.29^{c}

		3	31.17 ± 0.02^{a}
		4	30.5 ± 0.06^{b}

Results are reported as means ± S.D. Same letters in the same column refer to means not statistically different according to ANOVA and Tukey's test. TPC - total phenols yield referred to dry weight (DW) of stem; GAE - gallic acid equivalents.

The solvent constitution decides the type and quantity of phenolic compounds extracted from plant materials, and is one of the most important factors in an extraction process (Nayak, Dahmoune, Moussi, Remini, Dairi, Aoun, et al., 2015). Aqueous ethanol solution was widely used because it has low toxicity and good accessibility, and can easily dissolve phenolic compounds (Fang, Wang, Hao, Li, & Guo, 2015; H. Li, Deng, Wu, Liu, Loewen, & Tsao, 2012).

As it can be seen from Table 1, the extraction yields and TPC in stem extracts strongly depends on the concentration of ethanol, this could be due to the polarity of solvent, which was critical for the solubility of phenolic compounds (Simić, et al., 2016).

The effect of different concentrations of ethanol on total phenolic compounds yield was analysed, and other conditions were kept constant: 40:1 mL/g, 1 min, and 500 W for the ratio, time and power, respectively. As shown in Table VII, as the proportion of ethanol in hydroalcoholic solvent increased from 20 to 60 %, the TPC value was improved significantly ($p < 0.05$) from 26.34 ± 1.13 to 30.63 ± 1.41 mg GAE/g DW. However, the TPC value gradually decreased when the concentration of ethanol continued to rise. Therefore, 60 % ethanol was considered as the ideal concentration for further experiments.

Relatively high solvent volume could accelerate substance transfer and promote solubility, and then improve the extraction efficacy within a certain range as also reported by (Spigno & De Faveri, 2009). The impact of solvent/material ratio (S/M ratio) on phenol yield was investigated from 30:1 to 90:1 mL/g under these conditions (60 % ethanol, 1 min, 500 W). Table VII shows that the TPC value increased from 30:1 to 70:1 mL/g, and reached the peak (31.06 ± 3.12 mg GAE/g DW) at 70:1 mL/g, then it decreased (80:1 mL/g), and remained almost constant (80:1–

90:1 mL/g). We speculated that when the TPC value reached the peak at 70:1 mL/g, the substance transfer probably reached the equilibrium.

A sample of 1 g was extracted by 70:1 mL of 60 % ethanol for 1 min, with different levels of microwave power. As shown in table VII, the TPC value was improved with the increase of microwave power from 100 to 700 W, with a maximum of 38.08 - 0.20 mg GAE/g DW at 700 W.

As the irradiation power continued to rise, the TPC value decreased gradually, possibly because microwave power (> 700 W), indeed, higher microwave power could cause the degradation of phenolic compounds and affect negatively the extraction yield due to the thermal sensibility of these compounds. In general, the extraction yield increases proportionally with increasing microwave power up to a limit before the increase becomes insignificant or decline (Mandal & Mandal, 2010; Xiao, Han, & Shi, 2008). Hence, 700 W was chosen as the most efficient microwave power.

Table VII showed the influence of different extraction times on the TPC value when other conditions were fixed as: 60 % ethanol, 70:1 mL/g, 700 W. Firstly, the TPC value increased from 28.95 ± 0.31 to 31.17 ± 0.63 mg GAE/g DW with duration increasing from 1 to 3 min. When duration was extended to 4 min, the extraction efficiency decreased, and kept constant., Longer irradiation exposition without temperature control probably induced thermal degradation of phenolic compounds (Dahmoune, Spigno, Moussi, Remini, Cherbal, & Madani, 2014; B. Yang, Liu, & Gao, 2009).

III. Optimization microwave-assisted extraction (MAE)

Linear and interactive effect of independent variables over the response is examined through three-dimensional surface plots (3D). 3D plots were studied by varying two factors over a response while keeping the other two factors at a constant point. These were generated by plotting response in *Z*-axis and two varying factors in *X* and *Y* axis, respectively. These plots also help to interpret the optimum process conditions for maximum yield of response (Al-Dhabi, Ponmurugan, & Jeganathan, 2017).

The measured and predicted results for each trial for response are shown in Table VIII. The four independent variables and their corresponding levels were: X_1- Ethanol concentration (%) 40-60-80; X_2- Microwave power (W) 500-700-900; X_3- Irradiation time (min) 2-3-4 ; X_4- solvent/solid ratio (g/mL) 70-80-90.

Table .VIII. Box–Behnken design with the observed responses and predicted values for yield of total phenolic compounds (TPC) (mg GAE/g DW) of artichoke stem using MAE

Run	***Factors***				***Response (TPC yield in mg GAE/g DW).***	
	X1 Ethanol concentration (%, v/v)	**X_2 Microwave power (W)**	**X_3 Irradiation time (min)**	**X_4 Solvent / solid ratio (v:1mL/g)**	**Experimental**	**Predicted**
1	40	700	4	80	28.35	28.65
2	60	700	3	80	34.70	34.29
3	60	900	4	80	36.05	37.16
4	60	900	3	90	32.37	32.43
5	40	700	2	80	30.75	31.14
6	80	700	3	90	29.73	28.64
7	60	900	3	70	31.45	31.35
8	60	700	3	80	33.37	34.29
9	60	700	4	70	30.93	30.41
10	80	700	3	70	30.97	29.76
11	80	700	2	80	28.20	28.26
12	60	500	3	90	29.42	29.51
13	80	900	3	80	33.22	32.84
14	40	500	3	80	28.87	29.82
15	60	500	3	70	29.95	29.88
16	60	700	3	80	33.30	34.29
17	40	700	3	90	28.65	29.29
18	80	500	3	80	29.02	27.68
19	60	700	4	90	32.02	33.32
20	60	500	2	80	33.60	33.75
21	40	700	3	70	27.30	27.45
22	80	700	4	80	32.10	33.19
23	60	700	2	90	28.47	29.56
24	60	700	2	70	30.97	31.74
25	60	900	2	80	29.62	29.67
26	40	900	3	80	28.80	29.05

27	60	500	4	80	28.05	28.69

First, an analysis of variance (ANOVA) was performed to evaluate the effect of the variables, to identify possible interactions between them, and to assess the statistical significance of the model (Table IX).

Table.IX. Analysis of mean square deviation of the quadratic model terms (Eq. (3)) applied to the experimental values of total phenolic yields obtained with MAE extraction. df, degrees of freedom.

Source	**Sum of Squares**	**DF**	**F-Value**	***p*-value Prob > F**
Model	129.15	14	22.61	<.0001*
X_1- Ethanol concentration %	9.25	1	22.67	0.0005*
X_2- Microwave power (W)	13.21	1	32.40	0.0001*
X_3- Irradiation time (min)	2.89	1	7.09	0.0207*
X_4- sample/solvent (g/mL)	0.067	1	0.16	0.69
X_1X_2	4.56	1	11.19	0.005*
X_1X_3	9.92	1	24.31	0.0003*
X_1X_4	1.67	1	4.10	0.065
X_2X_3	35.88	1	87.93	<.0001*
X_2X_4	0.52	1	1.28	0.27
X_3X_4	3.31	1	7.88	0.01*
X_1^2	40.68	1	100.16	<.0001*
X_2^2	4.92	1	12.07	0.004*
X_3^2	6.75	1	16.54	0.0016*

X_4^2	20.95	1	51.36	<.0001*
Residual	0.99	15		
Lack of fit	3.65	10	0.58	0.76
Pure error	1.24	2		
R^2	0.96			
Adj-R^2	0.92			

With an asterisk (*) : Significant,

The statistical significance of the regression model was checked by *F*-test and *p*-value, the corresponding variables would be more significant if the *F*-value becomes greater and the *p*-value turns to smaller, therefore, the ANOVA in Table 4 showed that the fitted model was significant (F = 22.62, $p < 0.0001$). The non-significance of the lack-of-fit test (p = 0.76) verified the suitability of the selected model. The R^2 of 0.96 implied that 96 % of the variations of TPC value were attributed to the three independent variables. Besides, the adjusted R^2 value of 0.92 was close to the R^2 of 0.96, indicating that the observed values were correlated with the predicted values to a high degree.

All the above results revealed the validity of the model to predict the real correlations between the response value and independent variables.

This analysis provides information on the mathematical model that is generated from the experimental data. The extraction of both types of compounds is related to the experimental conditions by a second-order polynomial equation, with non-significant items being removed:

$$Y = 33.79 + 0.87X_1 + 1.04X_2 + 0.49X_3 + 1.06X_1X_2 + 1.57X_1X_3 + 2.99X_2X_3$$
$$+0.89X_3X_4 - 2.76X_1^2 - 0.96X_2^2 - 1.25X_3^2 - 1.98X_4^2 \quad \text{Eq. (8)}$$

Regarding the linear, quadratic and interaction effects on the response (Table IX), microwave power (X_2) followed by ethanol concentration (X_1) and irradiation time (X_3) were the most influent parameters, both respective linear and quadratic factors were highly significant ($p < 0.05$). However, the sample/solvent ration had the smallest effect on the response.

The interaction between the extraction variables; ethanol concentration and irradiation time (X_1X_3), ethanol concentration and irradiation time (X_1X_3), microwave power and irradiation time

(X_2X_3), irradiation time and sample/solvent ration (X_3X_4) were found to have a significant positive effects on the response. Positive signs indicate a direct relationship between the effect and the response variable, while negative signs refer to an inverse relationship

The effects of the independent variables and their mutual interaction on the extraction yield of phenolic acids can also be seen on three-dimensional response surface curves and contour plots shown in Fig.21. A–C. The plots were generated by plotting the response using the Z-axis against two independent variables while keeping the other two independent variables at their zero level (Hayat, Hussain, Abbas, Farooq, Ding, Xia, et al., 2009).

Fig.21. A–B depicts the interactions between the amount of ethanol concentration and each of both other factors (Microwave power, irradiation time) on the recovery of total phenolic content. The response value of the stem raised from 29 to 33.80 mg GAE/g DW when ethanol concentration increased from 40 to 65 %, and extraction power from 500 to 750 W and nearly reached a peak at the 65 % of ethanol concentration tested. In general, it was found that ethanol solution that ranged from 40 to 80 % had greater efficiency in the extraction of phenolics compared to pure ethanol (Hayouni, Abedrabba, Bouix, & Hamdi, 2007; Song, Li, Liu, & Zhang, 2011).

After that, additional ethanol concentration and extraction power caused negative effects according to Table .VIII, the recovery of TPC mainly depends on the ethanol concentration as its quadratic and linear effects were highly significant ($p < 0.01$), which result in a curvilinear increase in TPC yield for all the extraction powers and times tested (Fig. 21.A and B). Increase in the TPC suggests that the phenolic compounds are more soluble in ethanol/water, confirming the single-factor experiments results. Ethanol could increase the extraction yields and water could enhance swelling of cell material, increasing positively the contact surface area between plant matrix and solvent, resulting in increase of the extraction yield (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015; Hayat, et al., 2009).

As can be seen in Fig.21.C., an increase in microwave power enhanced the extraction yield at first, and then decreased it. This outcome is consistent with the data reported by Périno-Issartier, Zill e, Abert-Vian, and Chemat (2010) and Y. Li, Fabiano-Tixier, Abert-Vian, and Chemat (2012), who also observed a similar trend for the effect of microwave power on the MAE of

natural antioxidants from several plants. When a high microwave power was used, at long extraction times, it promotes the degradation of total phenolic contents.

As showed in fig.21.D. The increase of the liquid to solid ration from 70:1 to 85 mL/g improved greatly the yield of polyphenols which reached the peak at 82:1 mL/g, this result correlate with the preliminary tests after the decrease of the extraction of phenolic compounds.

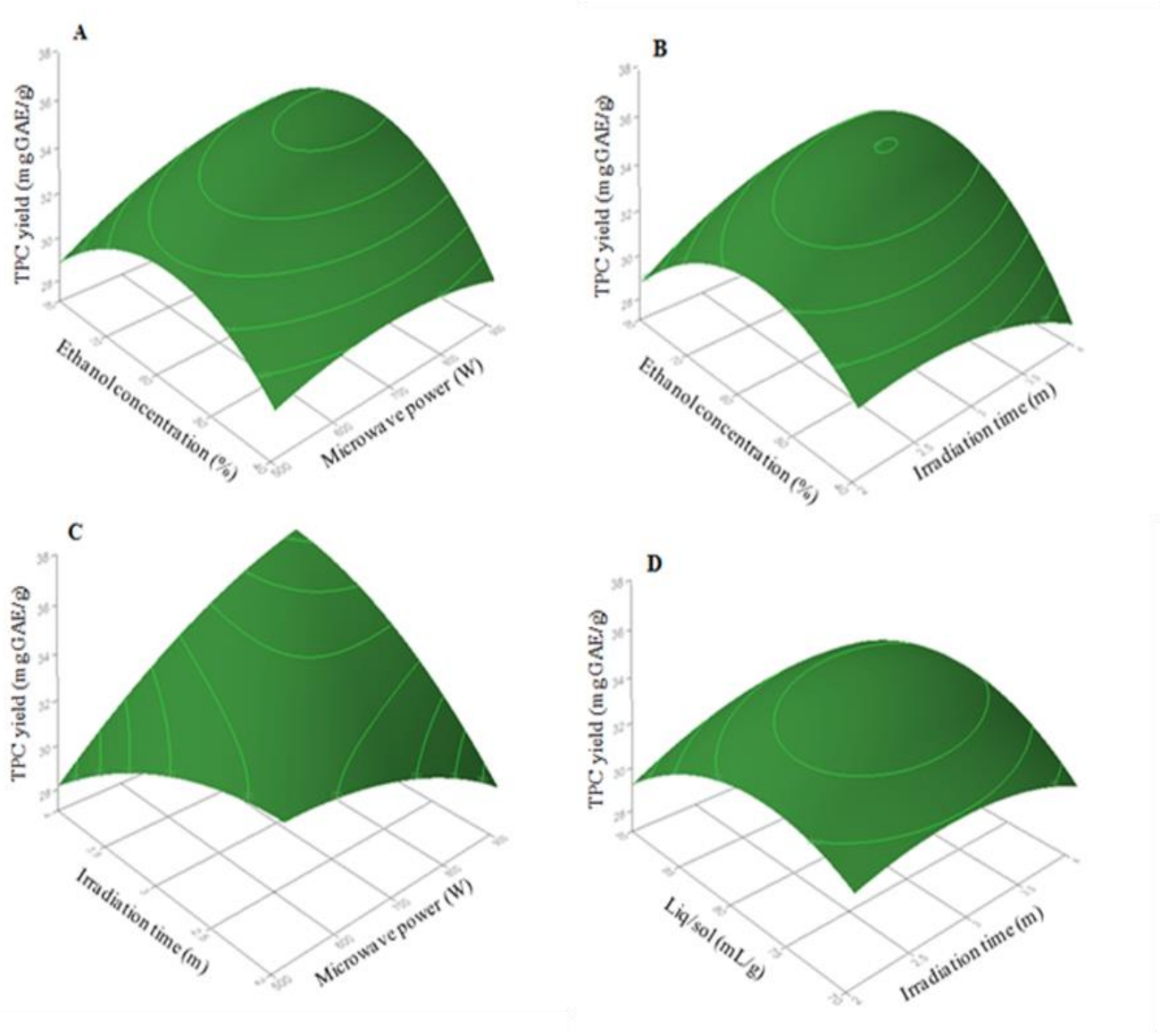

Figure .20**. Response surface analysis for the total phenolic yield from stem of artichoke (*Cynara scolymus L.)* with microwave assisted extraction with respect to ethanol concentration and microwave power (A); ethanol concentration and irradiation time (B); Irradiation time and Microwave power (C); liquid to solvent ration and irradiation time (D)**

This is consistent with mass transfer principles; the driving force during mass transfer is the concentration gradient between the solid and the liquid, which is greater when a higher

solvent to solid ratio is used. Similar results about the effect of solvent to solid ratio on the extraction of phenolic compounds were reported for date seeds by Al-Farsi and Lee (2008), Pinelo, Rubilar, Jerez, Sineiro, and Núñez (2005), and dried sage by Durling, Catchpole, Grey, Webby, Mitchell, Foo, et al. (2007).

IV.Validation of the models

On the basis of the RSM predictive models and values, the ideal MAE conditions for the extraction of TPC from stem were estimated to be: 72 % of ethanol, power of 900 W, irradiation time equal to 4 min, and solvent/solid ratio of 81:1 mL/g. Validation experiments were performed under the above mentioned optimal conditions to validate the adequacy of the models.

The mean yield value 36.10 ± 0.72 and (*n* =3) obtained from real experiments showed close correlations between the predicted and experimental values indicating the adequacy of the models to predict the optimal MAE conditions. Therefore, these conditions were recommended for future extractions of TPC, of Artichoke stem (Table X). These findings also justified the validity of the RSM model, and indicated that the model was adequate for the extraction process.

Table.X. Predicted and experimental values of TPC yield with microwave-assisted extraction (MAE) under optimised extraction conditions.

Run	Factors				Response (TPC yield in mg GAE/g DW)	
	X1 Ethanol concentration (%, v/v)	X_2 Microwave power (W)	X_3 Irradiation time (min)	X_4 Solvent / solid ratio mL/g	Experimental	Predicted
1	72	900	4	81:1	36.10 ± 0.72	37.43 ±1.26

V. Results of HPLC analysis

The HPLC DAD system has been used to determine the phenolic profile of the bract extracts obtained by three methods mentioned previously, 10 compounds have been identified (Fig 21.) The table X shows the different compounds corresponding to their corresponding peaks. The identification of different peaks have been achieved essentially on the basis of their pseudo molecular ion m/z and their fragmentation in the MS2.

Peak 2, 3,4 correspond to 3-*O*-caffeoyl quinic acid (neochlorogenic acid) , 5-O-caffeyoil quinic acid and 4-O-caffeoyl quinic acid (cryptochlorogenic acid). Fragmentation of the parent ion m/z 353 yielded product ions at m/z 191 and 179, with the quinic acid moiety representing the base peak and the caffeoyl moiety being present only with minor intensities, which is in agreement with literature findings (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford, Knight, & Kuhnert, 2005; Weisz, Kammerer, & Carle, 2009). Peaks 9, 10, were tentatively identified as 3,4-di-O-caffeoyl quinic acid, 3,5- di-O-caffeoylquinic acid respectively, on the basis of their fragmentation in the MS2 (Clifford, Knight, & Kuhnert, 2005; Schütz, Kammerer, Carle, & Schieber, 2004). Peaks 6, 7, and 11correspond to flavones following: Luteolin-rutinoside, Luteolin-hexoside (Luteolin-hexuronide), respectively (Zhishen, Mengcheng, & Jianming, 1999).

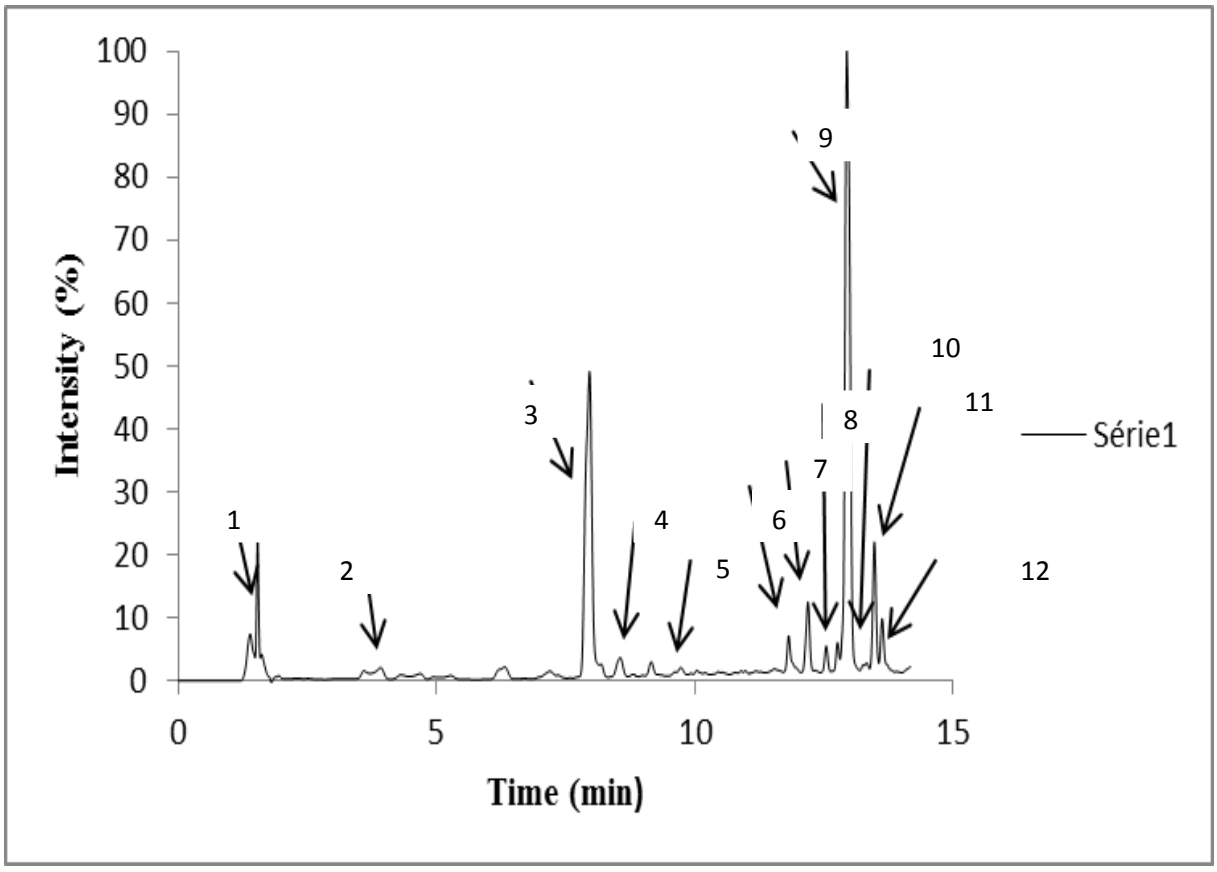

Figure .21. Representative chromatogram obtained by HPLC-DAD analysis of artichoke stem extracts obtained by MAE and CSE methods.

Table .XI. Metabolites identified by HPLC-DAD-ESI-MSn analysis of stem extracts.

HPLC fract	TR (min)	λmax	Molecular ion [M-H]-	Main fragment ESI- MSn	Identification
	1.5	205	133	115, 87	Malic acid
			191	111, 173	Citric acid
2	3.9	296sh, 327	353	191, 179	3-O-caffeyoilquinic acic
3	7.9	298sh, 325	353	191, 179 (10%)	5-O-caffeyoilquinic acid
4	8.5	289, 298, 324 mix	273	193, 229	
			353	173, 191, 179	4-O-caffeyoilquinic acid
5	9.6	311	337	191, 265, 161	191+ 146
6	11.7	252, 267, 337	593	285	Luteolin-rutinoside
7	12.1	205, 255, 345	447	285	Luteolin-hexoside
			461	285	Luteolin-hexuronide
8	12.5	245, 298sh, 328	515	353, 317, 299, 203	di-*O*-Caffeoylquinic acid isomer
			549	505, 365	
9	12.8	298 sh, 324	515	353, 335, 173	3,4 -di-*O*-Caffeoylquinic acid
10	12.9	298 sh, 324	515	353, 335, 191	3,5 -di-*O*-Caffeoylquinic acid
11	13.4	267, 337	445	269, 175	Apigenin-O- hexuronide
12	13.6	298sh, 325	515	353, 203, 299, 255	di-*O*-Caffeoylquinic acid isomer

VI.Comparison of MAE with conventional method

The efficiency of MAE was compared with maceration in several aspects, mainly in term of total phenolic content. The efficient of MAE process increased the total phenol yield by 93.77 % (18.63 ± 1.61 mg GAE/g DW for the maceration and 36.10 ± 0.72 mg GAE/g DW for MAE under optimised extraction conditions) in comparison with maceration extraction in a very short extraction time. In terms of the extraction duration, the best results were also achieved by the MAE with just 4 min (which conceded a 30-minute reduction in extraction time). In addition, MAE cost less solvent. Similar results were also reported in comparing MAE with conventional extraction techniques in extracting polyphenols from *Melastoma sanguineum* fruit by Zhao, Zhang, Li, Meng, and Li (2018) and from *Pistacia lentiscus* leaves by Dahmoune, Spigno, Moussi, Remini, Cherbal, and Madani (2014).

In fact, it is probable that the MAE may be directly correlated to the effect of microwaves on molecules by ionic conduction and dipole rotation. Note that these two extracts presented the same phenolic constituents (Figure .22. and Table XI), being dominated by caffeic acids derivatives, particularly of 3,5 -di-O-caffeoylquinic acid and 5-O-caffeyoilquinic acid, and the flavones luteolin-O-rutinoside luteolin-O-hexoside, luteolin-O-hexuronide and apigenin-O-hexuronide were detected as minor phenolic components. In general, this composition fits well with that previously described for extracts from artichoke origin, which also supports the theory that byproducts can also be valuable as a source of these phenolic components.

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VII. Effects of extraction methods on the antioxidant activity

The antioxidant activity of stem extracts were evaluated by the DPPH assay and compared to those of standard (ascorbic acid). DPPH / standard reduction was measured at 517 nm after 30 min of incubation with different concentrations of extract and standard.

The concentrations needed to reduce the DPPH radical by 50% (IC 50) were 0.041 and 0.067 mg/ml for the extracts obtained by MAE and CSE, respectively (Tables II and III, figure.3. in annexes). These values were greater than the IC 50 calculated for ascorbic acid (0.006 mg/ml).

However that obtained for MAE extracts showed a lower IC50 compared to the other extraction method, indicating higher antioxidant activity of MAE with higher scavenging of DPPH radicals compared to CSE methods.

Consistent with TPC values, MAE extracts also showed superior DPPH scavenging ability than the conventional extract. Again, even that the ability of the extracts was lower that of ascorbic acid, these results also consolidate the feasibility of using artichoke byproducts as a source of antioxidant compounds. As studied by Alaa A.Gaafar et al 2013 artichoke by-products presented a significant anti-oxidant activity, artichoke bracts and heart showed IC50 of (0.056 mg/ml and 0.077 mg/ml respectively).

The main reason for the improvement of MAE is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation in short extraction time and high efficiency in extracting. The absorption efficiency is largely related to the moisture content of the material; the water molecules convert the microwave energy into heat, and the result is a sudden rise in temperature inside the material. The quantity of release depends on the intensity and duration of application.

Materials and methods

(Part II-A-)

“Section II”

- ***The determination of dietary fiber distribution in different particles size of (Cynara scolymus L.)waste***

Materials and methods

Section II

I.Plant materiel

Before extraction, the samples were washed with distilled water and chopped into washers, and then were dried for about 48h at room temperature in a ventilated dark room, then ground with an electrical grinder (IKA model A11Basic, Germany), and sieved to obtain a thin powders of 125, 250, 500 and <500 ø respectively The powders was stored in airtight bags until use.

II.Chemicals and solvents

All solvents used were of analytical grade and purchased from Prolabo (CE), and all the collected samples were analysed for the TDF (SDF and IDF) by specific enzymatic degradation kits (Megazyme kit, GOPOD-format) according to the protocol briefly summarised as follows;

III. Determination of total dietary fiber:

III.1 Total Diatary Fiber (TDF)

Sample (1g) in duplicate

↓

40 mL buffer, ph 8.2 at 24°C

(MES/TRIS, 0.05 M)

↓

Stir sample beakers on magnetic

Stirrinfg for uniform dispersion of sample

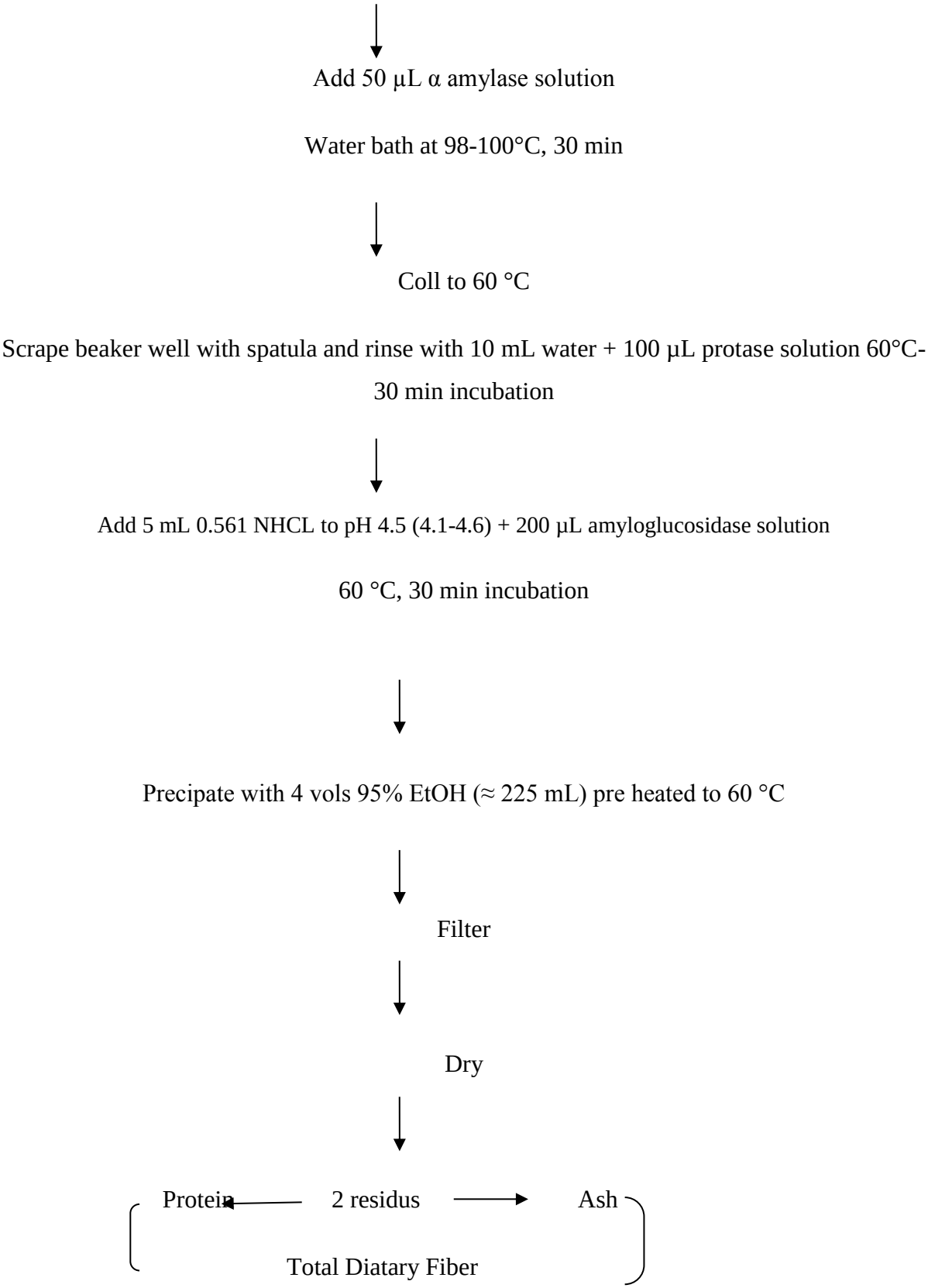

Figure .23. Analytical schema for the total diatary fiber determination procedure

III.2 Soluble and insoluble dietary fiber SDF/ IDF

Sample (1g) in duplicate in 600 ml beaker

↓

Add 40 ml MES-TRIS buffer, 0.05M each, pH 8.2 at 24 °C

↓

Add 50 µL heat stable α- amylase

↓

Water bath, 98-100 °C, 30 min

↓

Scrape beaker well with spatula, if necessary

Rinse with 10 ml water

↓

Add 100 µL protease (no pH adjustment)

↓

Water bath, 60 °C, 30 °C

↓

Add 5 ml 0.56 NHCL to Ph 4.1-4.8 and add 200 µL amyloglucosidase

(Leave beakers in 60 °C water bath until pH cheking/ adjusting step)

↓

Water bath, 60 °C, 30 min

↓

Filter through crucible

↓

Wash with 2 portions 10 mL water at 70 °C

(Use water to rinse beaker before washing residue)

↓ ↓

Filtrate + water washing Residue

↓ ↓ ↓

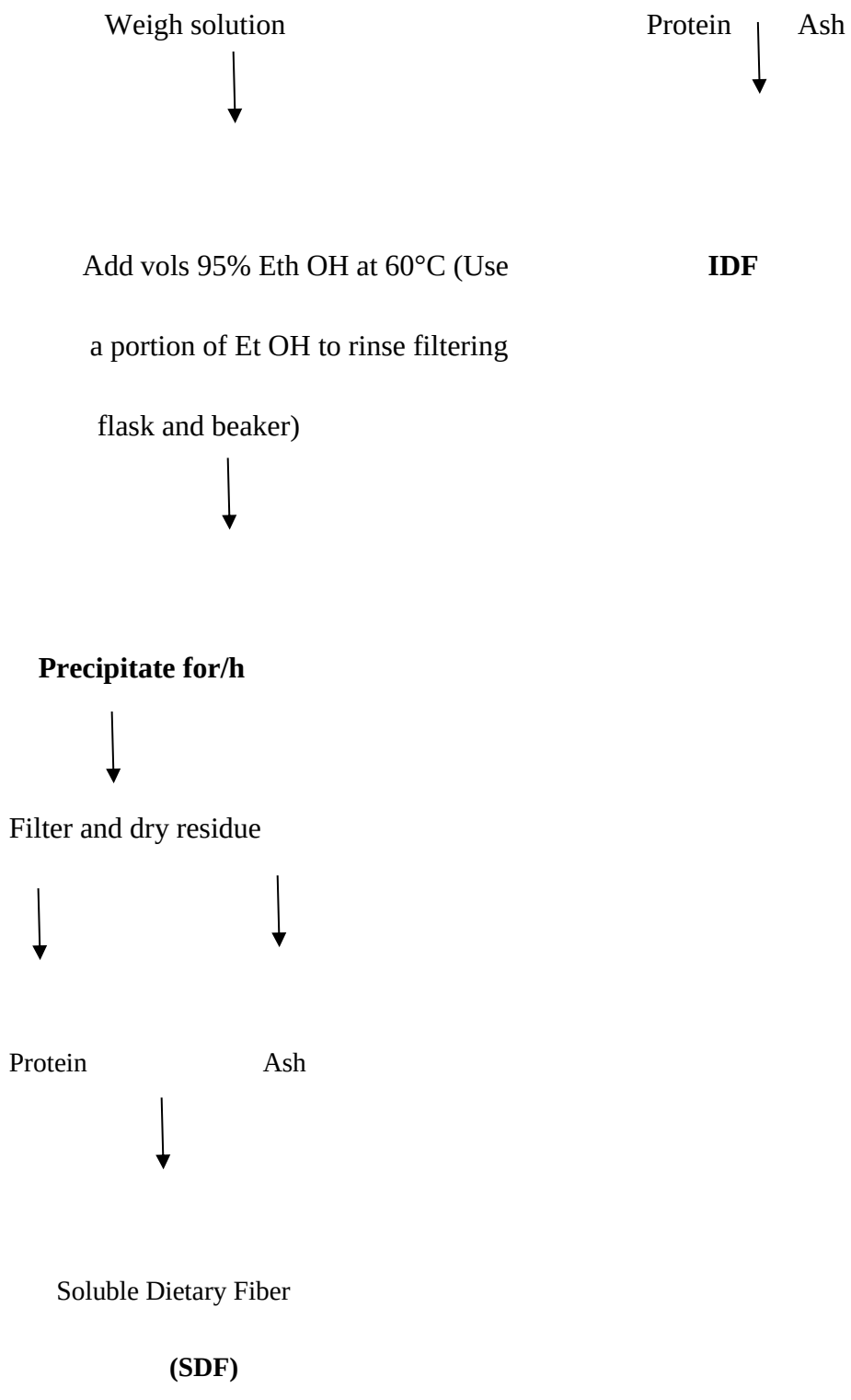

Figure .24. Analytical schema for soluble and insoluble diatary fiber determination procedure

III.3 Calculation

$$\textbf{Dietary Fiber \%} = \frac{\frac{R1+R2}{2} - P - A - B}{\frac{m1+m2}{2}} \times 100$$

$$R1 = residue\ weight\ 1\ from\ m1; R2 = residue\ weight\ 2\ from\ m2$$

$$m1 = sample\ weight\ 1; m2 = sample\ weight\ 2$$

$$A = ash\ weight\ from\ R1; P = protein\ weight\ from\ R2\ and\ \boldsymbol{B = blank}$$

$$= \frac{\boldsymbol{BR1 + BR2}}{\boldsymbol{2}} - \boldsymbol{BP - BA}$$

BR= blank residue

BP = blank protein from BR1

BA= blank ash from BR2

Results and discussion

(Part II-A-)

“Section II”

Results and discussion

"Section II"

I. Determination of dietary fibers) distribution of different particle sizes in stem powders (125ø, 250ø, 500ø and >500ø respectively)

Defining dietary fiber and measuring dietary fiber have always been challenging. From its start as crude fiber to its definition as dietary fiber. Historically, fiber was considered to comprise polysaccharides (degree of polymerization [DP] >10) that were resistant to digestion and absorption in the upper bowel, and could then be fermented in the gut. The chemical composition of the fiber included cellulose, hemicellulose, pectin (carbohydrates), and lignin(Korczak & Slavin, 2020).

The CODEX Alimentarius Commission defined dietary fiber as a group of carbohydrate polymer compounds with ten or more monomeric units (the footnote allows to include polymers with DP 3–9), which are neither digested nor absorbed in the human small intestine (Jones, 2014). Food products that naturally contain dietary fiber, such as cereals, fruits, vegetables, nuts, beans and seafood, but also breast milk and application of prebiotics in functional food are main sources of dietary fiber intake (Cai, Folkerts, Folkerts, Maurer, & Braber, 2020)

Dietary fiber means carbohydrate polymers with a degree of polymerization not lower than 3, which are neither digested nor absorbed in the small intestine. A degree of polymerization not lower than 3 is intended to exclude mono- and disaccharides. It is not intended to reflect the average degree of polymerization of a mixture. Dietary fiber consists of one or more of: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers obtained from food raw material by physical, enzymatic, or chemical means; synthetic carbohydrate polymers (Mudgil & Barak, 2013).

Besides its structure, dietary fiber has been described in terms of its solubility. Insoluble dietary fiber consists mainly of cellulose, hemicellulose, and lignin, while soluble dietary fiber consists mostly of noncellulosic polysaccharides, including pectin, gums, and .1 mucilages.1 Dividing fiber into soluble and insoluble fiber categories was based on differences in physiological effect;. Dietary fiber generally has properties such as: decrease intestinal transit time and

increase stool bulk; fermentable by colonic micro flora; reduce blood total and/or LDL cholesterol levels; reduce post-prandial blood glucose and/or insulin levels. More recently, other aspects of fiber – viscosity and fermentability, for example – were found to explain differences in physiological response in humans (Cai, Folkerts, Folkerts, Maurer, & Braber, 2020).

The total dietary fiber represent the insoluble and soluble dietary fiber respectively. As summarised in table XIV the highest DF outcomes were achieved with the >500ø particle size with 42.08 ± 0.86^{a} %, and the less interesting ones were reached 125ø with 32.55 ± 2.91^{c} %, the results obtained demonstrate that the fibre content of stem were proportional to the particles size in contrast to the phenolic compounds their distribution is inversely proportional to the particle size, which affirms that the distribution of both compounds is largely dependent on the particles size. In the case of bark tissues, the study of Chupinet al. (2015) show that the highest amount of total polyphenolsare extracted with the smallest particle size of maritime pine bark(40 mg GAE/g bark). (Bouras, Chadni, Barba, Grimi, Bals, & Vorobiev, 2015).

In the studies carried out by Claus, et al.(2015), who found that the other parts of the plant presented the fiber torer as follows; 44.23a ± 1.29 for the bracts, 13.07d ± 0.56 and 27.79c ± 1.14 for the Receptacle and Spikes respectively .

Table .XII. Results of dietary fiber distribution in different particles size of stem

Particle size	Insoluble dietary fiber	Soluble dietary fiber	Total dietary fiber
125 Ø	30,905	1,38125	32.55 ± 2.91[c]
	27,661875	3,134375	
	33,478125	0,926875	
250 Ø	35,67375	2,63625	37.97 ± 1.65[b]
	38,88125	1,219375	
	36,58875	0.97	
500 Ø	39,16375	4,341875	42.22 ± 1.20[a]
	39,725	0,926875	
	38.02	3.01	
< 500 Ø	43.41	0,64625	42.08 ± 0.86[a]
	41.69	-0,658125	
	42.72	-0,90125	

Conclusion

(Part II-A-)

Conclusion (Part II-A-)

Conclusion

Microwave-assisted extraction (MAE) is a feasible extraction technique. Various MAE techniques have improved the performance in plant extraction by coupling with respective modifications. Information collected from various reports and articles were reviewed and presented as useful guidelines in this article. Hence, suitable MAE techniques with specific operating conditions can be employed for plant extraction as the performance of MAE depends on the proper selection of extraction techniques, equipment setup and the extraction procedure with optimized operating parameters. As a concluding remark, MAE system is considered a promising technique for plant extraction.

The recovery of natural antioxidants or other health-promoting compounds from agro-industrial wastes is a topic of a growing interest in the field of applied biotechnology. In this context to the best of our knowledge this is the first report on the extraction of phenolic compounds from artichoke stem using MAE. RSM was successfully employed to optimize the extraction and several experimental parameters have been evaluated to determine the optimal conditions for the extraction.

The proposed method showed satisfactory linearity (correlation coefficients between 0.96 and 0.92). The applied second-order polynomial model gave a satisfactory description of the experimental data showing that the TPC yield was most affected by ethanol concentration and extraction power ($p < 0.05$).

Under the optimal conditions, the yield of stem was 36.10 ± 0.72 mg GAE/g DW, significantly higher than that of conventional extraction. According to the obtained results, it can be concluded that the microwave-assisted extraction was a promising technique for extracting phenolic compounds from stem, which might play important roles in improving extraction rate of antioxidant compared to traditional solid–liquid extraction.

Major chemicals in artichoke by-products include dietary fibers (i.e., fructan-type inulin, inositol, pectin cellulose, and hemicellulose) in the stem, root, and bracts, sesquiterpene lactones (i.e., cynaropicrin), fatty acids, minerals and polyphenolics (i.e., phenolic acids and flavonoids) abundant in leaves and seeds

The results obtained for the SDF, IDF and TDF reveals that their distribution is largely dependent on the particles size, the rich one was IDF .The highest value was attributed for the >500ø particle size with 42 %, and the less interesting ones were reached 125ø with 29 %.

(Part II-B-)

The Enrichment of a vegetable oil by antioxidants (the optimal MAE extract) of artichoke by-products, and the characterization of both enriched and control oils by HPLC analysis

Materials and methods

(Part II-B-)

Materials and methods (Part II-B-)

I.Materials

Sunflower oil purchased from a market, the extract obtained under the optimum conditions was evaporated under vacuum by rotary evaporator type Heidolph (Germany) in order to eliminate the extraction solvent and obtained an appropriate quantities of sample extract.

II. Chemicals and solvents

Sodium carbonate (Na_2CO_3), Folin–Ciocalteu phenol reagent were obtained from Prolabo (made in CE). Gallic acid was purchased from Biochem-chemopharma (UK) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) from Sigma Aldrich (Germany). All solvents used were of analytical grade and purchased from Prolabo (CE).

III. Incorporation of stem optimum extract in sunflower oil

The fortified sunflower oil was prepared according to the methodology reported by Dairi, et al. (2017). Appropriate weighed quantities of sample extract (Optimized MAE conditions) were dissolved in an appropriate volume of ethanol (50 %), in order to have a final concentration of 200 ppm. Then, 500 µL were added to sunflower oil (500 g) progressively under vigorous stirring for 30 min. Oil samples were stored in the dark until analysis. Oil sample without extract was considered the control sample.

III.1 Extraction of polyphenols from enriched oil

This procedure was adapted from the methodology described by Kalantzakis, Blekas, Pegklidou, and Boskou (2006). A sample of 2.5 g of oil was added to 5 mL of hexane (99 %) and 5 mL of methanol/water (6/4, v/v), then vortexed for 2 min and centrifuged at 3500 rpm for 10 min. The polar fraction (methanolic phase) was recovered, while the apolar phase (hexane) underwent exhaustion (depletion). The three fractions obtained were mixed and stored at 4 °C and protected from light. The same procedure was used with the control sample without artichoke waste extract.

III.2 Determination of total phenolic content

TPC of sunflower oil was determined according to Georgé *et al.* (2005) method. Briefly, 2.5 mL of diluted Folin-Ciolcateu reagent (diluted ten times 1/10) were added to the extracts. The mixture was incubated for 2 min at room temperature, and 2 mL of saturated sodium carbonate solution (75 g/L) were added. The mixture was incubated for 15 min at 50 ° C and finally cooled in a water-ice bath. The specific absorbance at 760 nm was immediately measured by a spectrophotometer (UV-mini 1240, Shimadzu, Japan). The TPC was expressed as milligram gallic acid equivalents per gram of dry weight (mg GAE/ g DW) basis.

III.3 DPPH• radical scavenging assay

As it was explained in the part II-A the radical scavenging activity of oils (enriched and control) was determined by using the DPPH• scavenging assay, according to the method optimized and described by (Neto, Marçal, Queirós, Abreu, Silva, & Cardoso, 2018), Briefly fifty microliters of the extract/standard were added to 250 µL of the DPPH solution. The mixture was placed in the dark for 30 min and the absorbance was then read at 517 nm.

III.4 UHPLC–DAD–ESI/MS^n analysis

The UHPLC-DAD-ESI/MS^n analysis was performed on an Ultimate 3000 (Dionex) apparatus equipped with an ultimate 3000 diode array detector and coupled to an ion trap mass spectrometer, following the general proceeding as previously described by (Wasli, Jelali, Silva, Ksouri, & Cardoso, 2018). The chromatographic system consisted of a quaternary pump, an autosampler, a degasser, a photodiode-array detector and an automatic thermostatic column compartment. Analysis was run on a HichromNucleosil C_{18} column (250 × 4.6 mm i.d., 5 µm particle diameter, end-capped) and its temperature was maintained at 30 °C. The mobile phase was composed of (A) acetonitrile and (B) 0.1% of formic acid (v/v), both degassed and filtered before use. The solvent gradient started with 90–70% of solvent B over 20 min, from70–40% of solvent B over 10 min, and from 40–0% of solvent B over 5 min.

For the HPLC analysis, each extract (50 mg) was dissolved in 5 mL in apropriate solvent. All samples were filtered through a 0.2 µm Nylon membrane (Whatman). The flow rate was 0.7 mL/min and split out at 200 µL/min to the MS. UV–Vis spectral data for all peaks were acquired in the range 250– 500 nm and chromatographic profiles were recorded at 280 nm. The mass spectrometer used was an Amazon SL (BrukerDaltonics) ion trap MS equipped with an ESI source.

Control and data acquisition were carried out with the Compass Data Analysis data system (BrukerDaltonics, Bremen, Germany). Nitrogen above 99% purity was used and the gas pressure was 520 kPa (75 psi). The instrument was operated in negative-ion mode with electrospray ionization (ESI) needle voltage set at 5.00 kV and an ESI capillary temperature of 200 °C. The full scan covered the mass range from m/z 70 to 700. Collision-induced dissociation (CID)-/MS and MS^n experiments were simultaneously acquired for precursor ions using helium as the collision gas with collision energy of 10–40 arbitrary units.

Results and discussion

(Part II-B-)

Results and discussion

(Part II-B-)

I. Quantification of phenolics in sun flower enriched oil

The extract obtained under the optimum conditions was tested in term of the efficiency by its incorporation in the sun flower oils. The TPC yield was examined and the results revealed the presence of phenolic compounds in the oil enriched with a concentration of 56.41 ± 2.03 µg/mL and its absence in the control oil (without extract) . These results confirmed that the extract has greatly improved the quality of the tested oil, by increasing its bioactive substances content, which in turn improves its nutritional quality, compared to the control oil which reveals no TPC.

II. Impact of the fortification of vegetable oils with artichoke byproduct extracts

As reported in table XV, figure.24, the characterization of the enriched (prepared from the optimum of MAE) and control oils was carried out by the HPLC analysis and by the DPPH test. The IC_{50} value (table XVI figure.25) was calculated for the enriched and not enriched oils (negative control), and ascorbic acid was used as a positive control.

The fortification of sunflower oil with MAE stem extracts (at 200 ppm) resulted in an improvement of the oils phenolic and antioxidant profiles. In fact, the UHPLC-DAD-ESI-MSn analysis of the ethanolic extract from the control sample oil showed no detectable phenolic constituent, while those fortified with extracts from artichoke byproduct contained 3,5 -di-O-Caffeoylquinic acid and 5-O-caffeyoilquinic acid, 4-O-caffeyoilquinic acid , di-O-Caffeoylquinic acid isomer , alike of their original extracts. This was also reflected in their antiradicalar ability towards DPPH, for which IC_{50} values were 0.193±0.02 mg/mL for MAE fortified oils, while % inhibition in the control sample was quite low .Hence, these results are in line with previous studies that demonstrate that the fortification of vegetable oils with phenolic-rich extracts can improve their antioxidant ability (Chougui, Djerroud, Naraoui, Hadjal, Aliane, Zeroual, et al., 2015).

Antioxidants naturally present in or added to oils exert beneficial effects by avoiding oil chemical alteration during heating. In the study conducted by Orozco, Priego-Capote, and Luque de Castro (2011) where they compared four types of oils as follow : Four different oils were selected to study the influence of antioxidants on the stability of the three target groups

of compounds present in the unsaponifiable fraction during simulated deep frying. Olive and sunflower oils were used as reference to assess the antioxidants' effect, as the former oil is characterized by(Chougui, et al., 2015) the natural presence of phenolic antioxidants, whereas sunflower oil loses them in the refining process. To obtain a VOO with a total phenol concentration of 400 μg/mL, expressed as caffeic acid

according to the Folin_Ciocalteu test, two different olive oils were mixed at the suitable proportion. On the other hand, pure refined sunflower was used as such and also enriched with natural antioxidants to compare their effect with that of a synthetic autoxidation inhibitor. The enrichment was as follows: (i) With an ethanolic extract of phenol compounds from an olive pomace residue using the protocol described by Giron et al.19 Enrichment was carried out up to a total phenols concentration of 400 μg/mL, expressed as caffeic acid. (ii) With 400 μg/mL of a synthetic autoxidation inhibitor (dimethylsiloxane). This additive (E900) was originally added to frying oils to prevent foaming, but it also possesses an oxidation inhibition activity by an uncertain mechanism.21 The speed and efficiency of the heating process depend on the temperature and quality of the oil. The heating temperature is usually between 150 and 190 _C, set for this experiment at 180 _C.

The results of the study revealed that the natural antioxidants such as phenolic compounds have demonstrated an antioxidant activity superior to that of synthetic antioxidants. Therefore, there is an increased trend to replace the latter with natural antioxidants. Enrichment with phenols protects edible oils against oxidation; that means better oil quality and prevention of the formation of toxic oxidation products such as cholesterol oxides.

Table .XIII. Metabolites identified by HPLC-DAD-ESI-MSn analysis of enriched sunflower oil.

HPLC fract	TR (min)	λmax	Molecular ion [M-H]-	Main fragment ESI- MSn	Identification
	1.5	205	133 191	115, 87 111, 173	Malic acid Citric acid
2	3.9	296sh, 327	353	191, 179	3-O-caffeyoilquinic acic
3	7.9	298sh, 325	353	191, 179 (10%)	5-O-caffeyoilquinic acid
4	8.5	289, 298, 324 mix	273 353	193, 229 173, 191, 179	4-O-caffeyoilquinic acid
5	9.6	311	337	191, 265, 161	191+ 146?
6	11.7	252, 267, 337	593	285	Luteolin-rutinoside
7	12.1	205, 255, 345	447 461	285 285	Luteolin-hexoside Luteolin-hexuronide
8	12.5	245, 298sh, 328	515 549	353, 317, 299, 203 505, 365	di-*O*-Caffeoylquinic acid isomer
9	12.8	298 sh, 324	515	353, 335, 173	3,4 -di-*O*-Caffeoylquinic acid
10	12.9	298 sh, 324	515	353, 335, 191	3,5 -di-*O*-Caffeoylquinic acid
11	13.4	267, 337	445	269, 175	Apigenin-O- hexuronide
12	13.6	298sh, 325	515	353, 203, 299, 255	di-*O*-Caffeoylquinic acid isomer

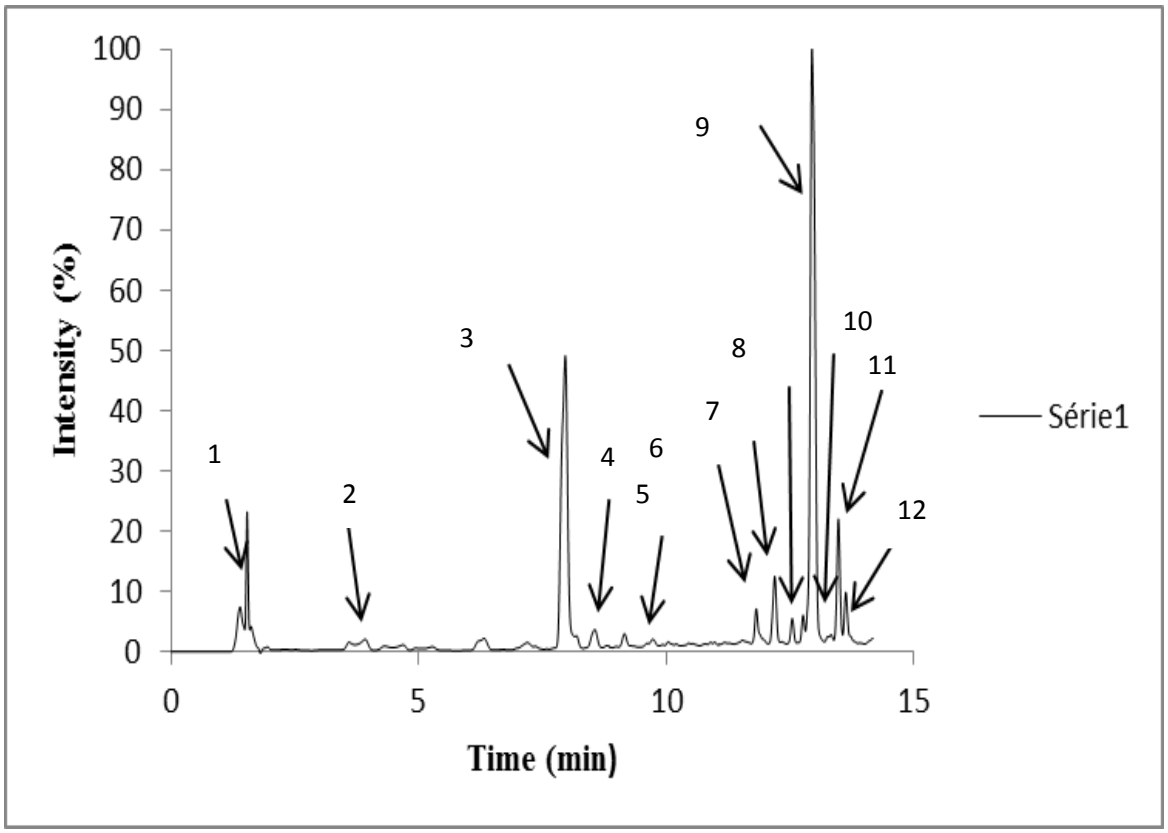

Figure .22. Representative chromatogram obtained by HPLC-DAD analysis of enriched sunflower oil

Table. XIV.Results of DPPH radicals (IC50) experiments for the sun flower enriched oil

fd	$V_{diluição}$ (mL)	$V_{sol-mãe\ padrão}$ (mL)	$[Extrato]_{dil}$ (mg/mL)	V mR (mL)	V extrato adcionado	$[extrato]_{poço}$ (mg/mL)	Abs 517 nm			% inibição	IC50 (mg/mL)	IC50 (ug/mL)
							R1	R2	Média			
12	0,6	0,050	0,6	0,3	0,05	0,093	0,551	0,548	0,550		0,193	192,90
10	0,5	0,050	0,7			0,111	0,465	0,503	0,484	27,0		
8	0,4	0,050	0,8			0,139	0,435	0,406	0,421	36,6		
6	0,3	0,050	1,1			0,185	0,346	0,351	0,349	47,4		
4	0,2	0,050	1,7			0,278	0,216	0,156	0,186	71,9		
2	0,2	0,100	3,3			0,556	0,111	0,126	0,119			

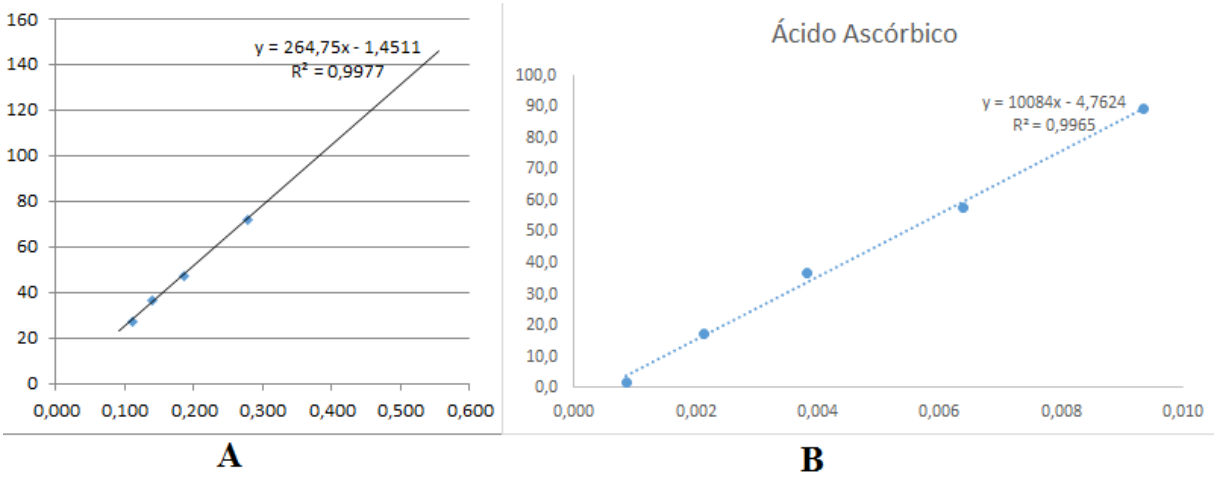

Figure .23. The (IC 50) obtained for; enriched sunflower oil (A) and ascorbic acid (B) .

Conclusion

(Part II-B-)

Conclution

Optimum stem extract presented significant levels of phenolic compounds that play an important role against oxidation as shown by the DPPH test and it can replace the BHA widely used in food preservation.

Utilization of stem for extraction of beneficial phytonutrients such as phenolic antioxidants not only provides health benefits, but also adds value to the waste generated by the artichoke processing industries.

In this present study, we showed that the enrichment of sunflower oil by stem extracts improved the antioxidant activity of the final product by increasing the scavenging effect toward free radicals and inhibiting the phospholipid peroxidation more effectively.

The enriched oil had a new composition of phenolic compounds from stem extracts, better antioxidant properties and may act against free radical attacks occurring during lipid digestion through several mechanisms of action.

The results obtained revealed that the enriched oil exhibits a better anti-oxidant activity compared to the control oil (not enriched) by the DPPH test, and the characterization of the oils by HPLC analysis confirm the presence of the same profile phenolic in the stem extracts and in the enriched oil however its absence in the control oil

General conclusion

Different processes based on novel extraction techniques and white biotechnology have been developed and optimized to obtain the maximal benefits from artichoke. biowastes to include mostly the recovery of its bioactive phyto constituents and production of value-added products. These approaches include optimization of extraction parameters, application of metabolomic-based quality assurance techniques, and fermentation of fibers. Contrary to the simple structure of immature flower capitulum, which aids in the release of phenolic compounds, other plants parts are of complex matrices rich in protein, fatty acids, and fibers. Novel extraction techniques, including pre-treatment, enzyme-, ultrasound- and microwave-assisted extractions are thus needed to aid in the release of phyto constituents, improved yield, and hydrolysis of polysaccharides into monomers that are further fermented to bioethanol.

Microwave-assisted extraction (MAE) is a feasible extraction technique. Various MAE techniques have improved the performance in plant extraction by coupling with respective modifications. Information collected from various reports and articles were reviewed and presented as useful guidelines in this article. Hence, suitable MAE techniques with specific operating conditions can be employed for plant extraction as the performance of MAE depends on the proper selection of extraction techniques, equipment setup and the extraction procedure with optimized operating parameters. As a concluding remark, MAE system is considered a promising technique for plant extraction.

The recovery of natural antioxidants or other health-promoting compounds from agro-industrial wastes is a topic of a growing interest in the field of applied biotechnology. In this context to the best of our knowledge this is the first report on the extraction of phenolic compounds from artichoke stem using MAE. RSM was successfully employed to optimize the extraction and several experimental parameters have been evaluated to determine the optimal conditions for the extraction.

The proposed method showed satisfactory linearity (correlation coefficients between 0.96 and 0.92). The applied second-order polynomial model gave a satisfactory description of the experimental data showing that the TPC yield was most affected by ethanol concentration and extraction power ($p < 0.05$).

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phenolic compounds from stem, which might play important roles in improving extraction rate of antioxidant compared to traditional solid–liquid extraction.

Microwave technique gave a significant increase in extraction yield with various advantages including: reduced extraction time, the use of less extraction solvent for a particular bioactive phytochemical when conditions are optimized and better extraction yield.

Major chemicals in artichoke by-products include dietary fibers (i.e., fructan-type inulin, inositol, pectin cellulose, and hemicellulose) in the stem, root, and bracts, sesquiterpene lactones (i.e., cynaropicrin), fatty acids, minerals and polyphenolics (i.e., phenolic acids and flavonoids) abundant in leaves and seeds.

Therefore, novel uses have been proposed for globe artichoke and its by-products, including the recovery of these valuable chemicals for further production of value-added products (i.e., food additives, biofuels, and agrochemicals). Optimization methods to improve the exploitation of these chemicals include the application of novel ultrasound- and enzyme-assisted extraction techniques, in addition to fermentation processes for the efficient and eco-friendly use of artichoke biowastes. Such practices are developed by researchers in the search for other renewable resources for energy, medications, and food have yet to be fully focused upon in the case of artichoke.

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The enriched oil had a new composition of phenolic compounds from stem extracts, better antioxidant properties and may act against free radical attacks occurring during lipid digestion through several mechanisms of action. However, various kinds of interactions between antioxidants in a complex mixture may occur and thus can affect the total antioxidant capacity of the mixture. For this, it is important to choose the best combination of antioxidants when designing functional foods. Further studies are needed to evaluate the bio absorption, bioavailability and interactions between these compounds present in enriched sunflower oil after consumption.

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Annexes

Annexe 1

Table. I. Artichoke world production in 2016

Rank	Country/Region	Artichoke production (tonnes)
1	Italy	365,991
2	Egypt	236,314
3	Spain	185,796
4	Peru	108,801
5	Argentina	107,257
6	Algeria	97,139
7	China	85,532
8	France	45,914
9	United States	44,720
10	Morocco	44,144
11	Turkey	36,368

Annex 2

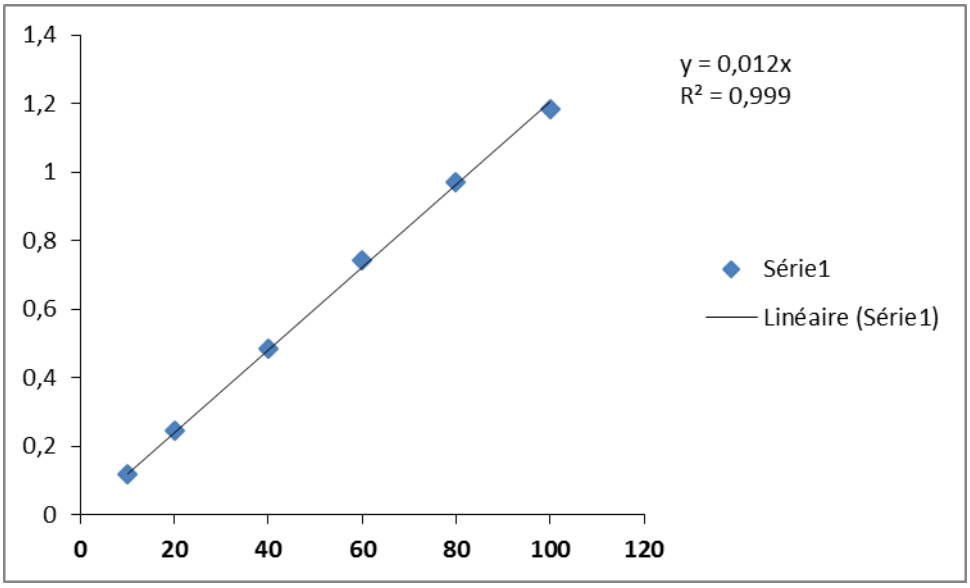

Figure.1. ***Calibration curve of Gallic acid***

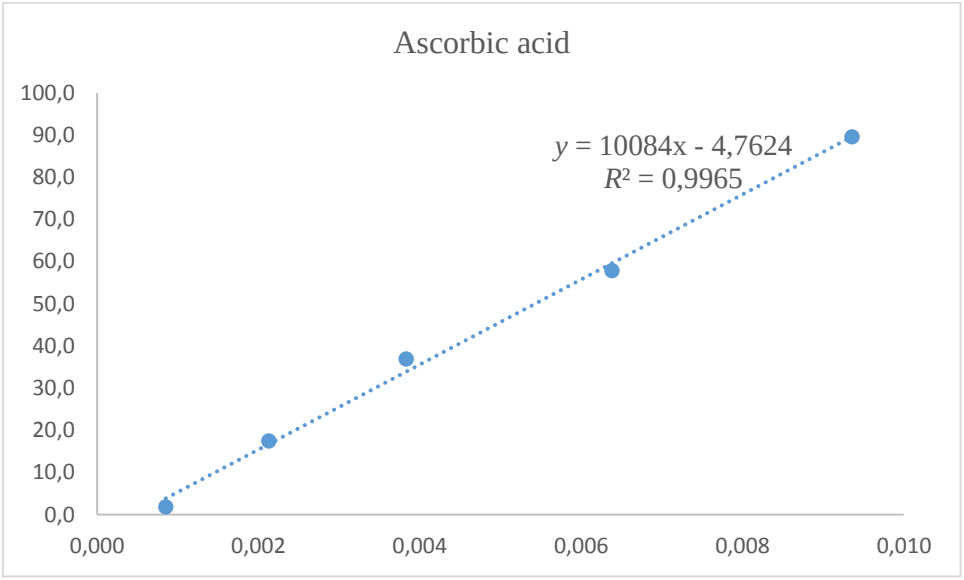

Figure.2. ***Calibration curve of the standard ascorbic acid.***

Table.II. Results of DPPH radicals (IC50) experiments for the standard ascorbic acid.

$V_{padrão}$ (mL)	$V_{sol\text{-}mãe\ padrão}$ (mL)	$[AA]_{dil}$ (mg/mL)	V mR (mL)	V AA adcionado	$[AA]_{poço}$ (mg/mL)	Abs 517 nm				% inibição	IC50 (mg/mL)	IC50 (ug/mL)
						R1	R2	R3	Média			
1	0,000	0	0,300	0,05	0,000	0,679	0,692	0,698	0,690		0,006	5,52
	0,020	0,005			0,001	0,684	0,683	0,674	0,680	1,4		
	0,050	0,0125			0,002	0,597	0,585	0,545	0,576	16,5		
	0,090	0,0225			0,004	0,516	0,481	0,446	0,481	30,3		
	0,150	0,0375			0,006	0,253	0,244	0,434	0,310	55,0		
	0,220	0,055			0,009	0,074	0,072	0,069	0,072	89,6		

Table .III. Results of DPPH radicals (IC50) experiments for the MAE

fd	$V_{dilutin}$ (mL)	$V_{norme\ sol\text{-}mère}$ (mL)	[Extrait] dil (mg/mL)	V mR (mL)	V extract added	extract]well (mg/mL)	Abs 517 nm			% inibição	IC50 (mg/mL)	IC50 (ug/mL)
							R1	R2	Média			
12	0,6	0,050	0,083	0,3	0,05	0,014	0,647	0,644	0,646	6,7	0,041	41,00
10	0,5	0,050	0,100			0,017	0,627	0,633	0,630	9,0		
8	0,4	0,050	0,125			0,021	0,62	0,604	0,612	11,6		
6	0,3	0,050	0,167			0,028	0,512	0,492	0,502	27,5		
4	0,2	0,050	0,250			0,042	0,358	0,304	0,331	52,2		
2	0,2	0,100	0,500			0,083	0,273	0,262	0,268			

Table .IV. Results of DPPH radicals (IC50) experiments for CSE

fd	$V_{diluição}$ (mL)	$V_{sol\text{-}mãe\ padrão}$ (mL)	$[Extrato]_{dil}$ (mg/mL)	V mR (mL)	V extrato adcionado	$[extrato]_{poço}$ (mg/mL)	Abs 517 nm			% inibição	IC50 (mg/mL)	IC50 (ug/mL)
							R1	R2	Média			
12	0,6	0,050	0,167	0,3	0,05	0,028	0,574	0,545	0,560	19,1	0,067	66,57
10	0,5	0,050	0,200			0,033	0,534	0,511	0,523	24,5		
8	0,4	0,050	0,250			0,042	0,469	0,477	0,473	31,6		
6	0,3	0,050	0,333			0,056	0,398	0,398	0,398	42,5		
4	0,2	0,050	0,500			0,083	0,264	0,259	0,262	62,2		
2	0,2	0,100	1,000			0,167	0,145	0,165	0,155			

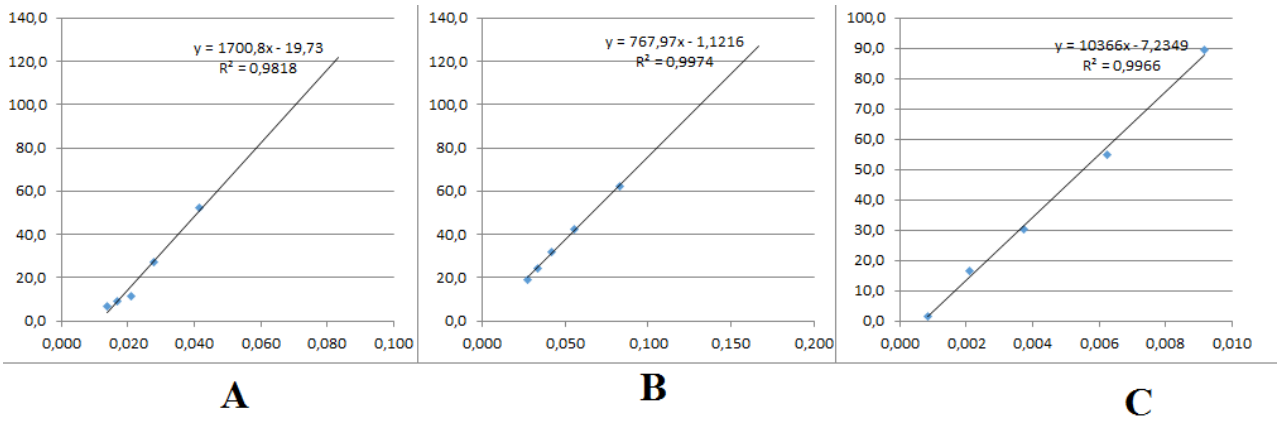

Figure .3. The (IC 50) obtained for; MAE (A), CSE (B) and ascorbic acid (C)

Annex 3

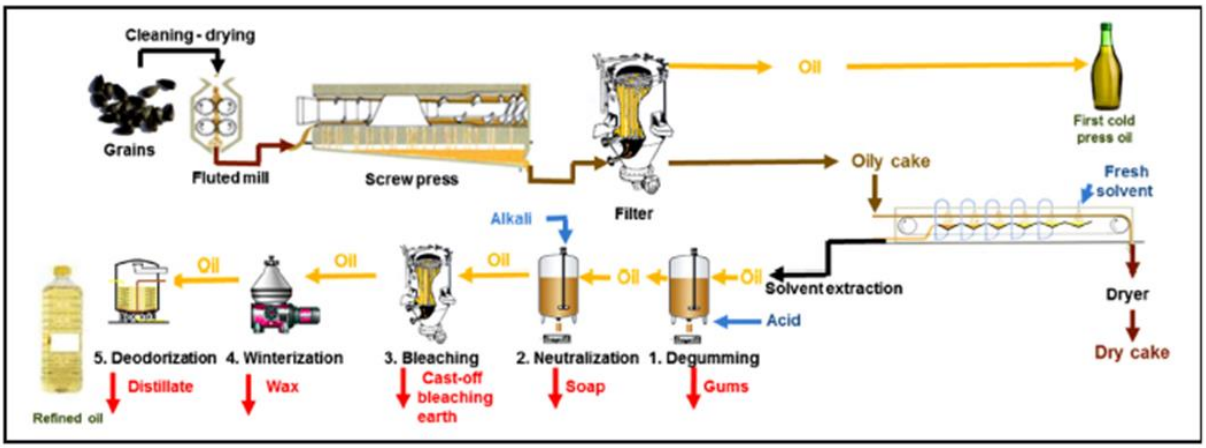

Figure.4. Extracting and refining steps of sunflower oil.(Orozco, Priego-Capote, & Luque de Castro, 2011)

ABSTRACT. The present study reports on the optimization of phenolic compounds extraction and dietary fiber distribution from artichoke (*Cynara scolymus L.*) waste, completed by the development of a functional enriched edible oil with potential health promoting effects. Box–Behnken Design (BBD), a widely used form of response surface methodology (RSM), was applied to investigate the effect of process variables on the microwave-assisted extraction (MAE) for the recovery of total phenolic compounds (TPC). Independent variables including ethanol concentration (%), microwave power (W), irradiation time (min), solvent-to-material ratio (mL/g) for MAE were studied. The statistical analysis revealed that the optimal MAE conditions were obtained with 72 % ethanol as extraction solvent, 81:1 mL/g of solvent/solid ratio, 4 min and 900 W for irradiation time and power, respectively. Maximum predicted TPC recovery under the optimized conditions was 37.43 ± 1.26 mg GAE/g DW, which was close to the experimental value 36.10 ± 0.72, indicating suitability of the employed model and the success of RSM in optimizing the extraction conditions. The both TPC and antioxidant activity determined by the DPPH test confirmed the efficiency of MAE methods when compared with the conventional method. The studied edible oil enriched with the optimized extract had a content of phenolic compounds equivalent to 56.41 ± 2.03 µg/mL compared to the control oil.
The results obtained revealed that the enriched oil exhibits a better anti-oxidant activity compared to the control oil (not enriched) by the DPPH test, and the characterization of the oils by HPLC analysis confirm the presence of the same profile phenolic in the stem extracts and in the enriched oil however its absence in the control oil.The results obtained for dietary fiber reveals that their distribution is largely dependent on the particles size.

***Keywords**: Artichoke byproducts, Optimization, Microwave assisted extraction, Phenolic compounds, Enrichment, oil, dietary fiber.*

RÉSUMÉ. La présente étude rend compte de l'optimisation de l'extraction des composés phénoliques et de la distribution des fibres alimentaires à partir des déchets d'artichauts (Cynara scolymus L.), complétée par la mise au point d'une huile comestible enrichie en fonctions, dont les effets potentiels sur la santé sont considérables. Le Box-Behnken Design (BBD), une forme largement utilisée de méthodologie de surface de réponse (RSM), a été appliqué pour étudier l'effet des variables du processus sur l'extraction assistée par micro-ondes (MAE) pour la récupération des composés phénoliques totaux (TPC). Des variables indépendantes, dont la concentration d'éthanol (%), la puissance des micro-ondes (W), le temps d'irradiation (min), le rapport solvant/matière (mL/g) pour l'EMA ont été étudiées. L'analyse statistique a révélé que les conditions optimales d'EMA étaient obtenues avec 72 % d'éthanol comme solvant d'extraction, 81:1 mL/g de rapport solvant/solide, 4 min et 900 W pour le temps et la puissance d'irradiation, respectivement. La récupération maximale prévue du PTC dans les conditions optimisées était de 37,43 ± 1,26 mg d'EAG/g de DW, ce qui était proche de la valeur expérimentale de 36,10 ± 0,72, indiquant la pertinence du modèle employé et le succès du RSM dans l'optimisation des conditions d'extraction. Le PTC et l'activité antioxydante déterminés par le test DPPH ont confirmé l'efficacité des méthodes d'EMA par rapport à la méthode classique. L'huile alimentaire étudiée enrichie avec l'extrait optimisé avait une teneur en composés phénoliques équivalente à 56,41 ± 2,03 µg/mL par rapport à l'huile témoin. Les résultats obtenus ont révélé que l'huile enrichie présente une meilleure activité antioxydante que l'huile témoin (non enrichie) par le test DPPH, et la caractérisation des huiles par analyse HPLC confirme la présence du même profil phénolique dans les extraits de tiges et dans l'huile enrichie, mais son absence dans l'huile témoin. Les résultats obtenus pour les fibres alimentaires révèlent que leur distribution dépend largement de la taille des particule.

***Mots clés:** Sous-produits d'artichaut, Optimisation, Extraction assistée par micro-ondes, Composés phénoliques, Enrichissement, huile, fibre alimentaire*.

ملخص. تشير الدراسة الحالية إلى الاستفادة المثلى من استخلاص المركبات الفينولية وتوزيع الألياف الغذائية من نفايات الخرشوف (Cynara scolymus L.) ، الذي تم استكماله بتطوير زيت صالح للأكل غني بوظائف ، وآثارها الصحية المحتملة كبيرة .تم تطبيق Box-Behnken Design (BBD) ، وهو شكل مستخدم على نطاق واسع لمنهجية سطح الاستجابة (RSM) ، لدراسة تأثير متغيرات العملية على الاستخراج بمساعدة الميكروويف (MAE) من أجل الاستعادة إجمالي المركبات الفينولية .(TPC) تمت دراسة المتغيرات المستقلة بما في ذلك تركيز الإيثانول) ٪ (، طاقة الميكروويف (W) ، وقت التشعيع) دقيقة (، نسبة المذيب / المادة)مل / جم (لـ . .EMA أظهر التحليل الإحصائي أنه تم الحصول على ظروف EMA المثلى باستخدام72 ٪ من الإيثانول كمذيب استخلاص ، ونسبة 1 :81 مل / جم مذيب / صلب ، و 4 دقائق و 900 وات للوقت والطاقة .التشعيع ، على التوالي .كان الحد الأقصى المتوقع لاسترداد PTC في ظل الظروف المثلى هو 37.43 1.26 ±مجم / EAG جم DW ، والذي كان قريبًا من القيمة التجريبية البالغة 0.72 ± 36.10 ، مما يشير إلى ملاءمة النموذج .الموظف ونجاح RSM في تحسين ظروف الاستخراج .أكد PTC والنشاط المضاد للأكسدة الذي حدده اختبار DPPH فعالية طرق EMA مقارنة بالطريقة التقليدية .كان لزيت الطعام المدروس المخصب بالمستخلص الأمثل محتوى فينولي يعادل 2.03 ± 56.41 ميكروغرام / مل مقارنة بزيت التحكم.

أظهرت النتائج التي تم الحصول عليها أن الزيت المخصب يُظهر نشاطًا مضادًا للأكسدة أفضل من زيت التحكم) غير المخصب (بواسطة اختبار DPPH ، ويؤكد توصيف الزيوت بواسطة تحليل HPLC وجود نفس المظهر الفينولي في مستخلصات السيقان و في الزيت المخصب ، لكن غيابه في زيت التحكم .تظهر النتائج التي تم الحصول عليها للألياف الغذائية أن توزيعها يعتمد إلى حد كبير على حجم الجزيئات..

الكلمات المفتاحية :منتجات الخرشوف الثانوية ، التحسين ، الاستخلاص بمساعدة الميكروويف ، المركبات الفينولية ، الإثراء ، الزيت ، الألياف الغذائية.