République Algérienne Démocratique et Populaire

Ministère de l'enseignement Supérieur et de la Recherche scientifique

Université A. MIRA - Bejaia

Faculté des Sciences et de la Nature et de la Vie

Département de Sciences Alimentaires

Filière : Sciences Alimentaires

Option : Biotechnologies, agro ressources, aliment et nutrition

Réf :.....

Mémoire de Fin de cycle

En vue de l'obtention du diplôme

MASTER

Thème

Optimisation de l'extraction assistée par micro-ondes des composés phénoliques des graines de tomates par la méthodologie de surface de réponse

Présenté par :

HAMZI Rebiha & GUENFIS Amina

Soutenu le : **16 Juin 2015**

Devant le jury composé de :

Dr BOULEKBACHE L	MCA	Presidente
M^{me} GUEMGHAR H	MAA	Promotrice
Dr HAMRI S	MCB	Examinatrice
M^{elle} GUEMGHAR M	Doctorante	Invitée

Année universitaire : 2014 / 2015

Remerciements

Au terme de ce modeste travail, nous tenons à exprimer nos remerciements les plus sincères tout d'abord au bon DIEU de nous avoir donné le courage, la santé et toute la patience qui nous ont été utiles tout au long de notre parcours.

Nos remerciements vont également à:

Les membres du jury, La présidente du jury Dr BOULEKBACHE Lila de nous avoir fait l'honneur de présider le jury. Egalement Dr. HAMMRI Sabrina d'avoir accepté d'examiner notre travail.

Notre promotrice M^me^ GUEMGHAR Hayate d'avoir accepté de nous encadrer, pour son suivi, ses conseils, ses orientations et sa précieuse aide.

M^elle^ GUEMGHAR Menana pour son aide.

A toutes les personnes qui ont contribué de près ou de loin à la réalisation de ce mémoire

Dédicaces

Je dédie ce modeste travail :

A la mémoire de mon père

A ma chère mère

A mes chères frères et sœurs

A mes cousines : Habiba ,Ilhem et Sihem

A mes amies:Amel, Sabah, Meriama , Sabrina, fatima , Melaaz, Nassima, Nadjiba, lynda, souhila ,karima .

A toute la famille HAMZI

A ma binôme : Amina et à toute sa famille

A toute la promotion Biotechnologies 2015

Rebiha

Dédicaces

Je dédie ce modeste travail

A mon adorable très cher père pour son affection, sacrifices et ses efforts afin me voir réussir, je lui serai reconnaissante tout le reste de ma vie

A mon trésor, source intarissable de tendresse, ma chère mère, Nedjima

A mes très chers grand parents Djedi said et nana houria ,zahra

A mes deux chers frère Fayçal et Younes

A mes oncles Mustapha, Hakim et sa femme

A mes tendres tantes Amti Houria, Fatima et Samia et leur famille

A Mes oncles maternels Fatah , Rabah et Zaher

A mes chère cousin et cousines

A mon très cher binôme Rebiha, ensemble nous avons pu faire et achever ce travail

A mes très chers amis Samia que j'aime énormément, Aziza, Souhila et khadidja

Amina

List of abbreviations

ANOVA: Analysis Of Variance

Aw: Activity of water

d.d.l: degrees of freedom

DO: Optical density

DPPH: 2,2-Diphenyl-picrylhydrazyl

Dw: dry weight

DW: dry weight

GAE: Gallic Acid Equivalent

M: Molar

MAE: Microwave Assisted Extraction

RSM: Response Surface Methodology

TP: Tomato pomace

TPC: Total Phenolic Compounds

v/v: volume/ volume

W: Watt

µMTEAC : micromolair Trolox equivalent antioxidant capacity

List of figures

List of table

Table of contents

Chapter III: Response surface methodology

The experimental part

Chapter IV: Materials and methods

Chapter V: Results and discussion

Introduction

The natural phenolic compounds have received increasing interest in the last years, since a great amount of them can be found in plants and consumption of vegetables and beverages with a high level of such compounds may reduce the risk of development of several diseases due to their antioxidant power .The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects(Kähkönen et al., 1999).

Tomato (*Lycopersicon esculentum L.*) is the second most important vegetable crop next to potato worldwide, with annual production at 100 million tons fresh fruit produced in 144 countries. The industrial processing of tomato leads to by-products, namely tomato seeds and peels, representing 10–40% of total processed tomatoes These by-products represent a major disposal problem for the industry, intended mainly for animal feed or fertiliser, whereas they usually constitute a promising source of compounds that can be used for their nutritional properties and biological potential (Kalogeropoulos et al., 2012; Strati and Oreopoulou, 2011).

Microwave-assisted extraction (MAE) is one of the novel extraction techniques which give better extraction yield, enhance the quality of extracts while decreasing the extraction time and the solvent consumption in comparison to conventional techniques(Karabegović et al., 2013).

MAE allows fast extractions, without the degradation of thermolabile compounds, with considerable savings in time and energy, and this technique is already used for the extraction of bioactive substances which are of interest for the food and pharmaceutical industry .Also, another important advantage of the microwave is its applicability in a laboratory, on a pilot and industry scale. Except for the extraction techniques, obtaining bioactive compounds from plant materials depends on many factors (the nature of the plant material, solvent properties, temperature, the plant material to solvent ratio, pressure, the duration of the process and other parameters that could affect the extraction process) (Karabegović et al., 2013).

The individual and combined effects of these factors on the efficiency of the isolation procedure can be estimated by statistical methods such as response surface

methodology (RSM). RSM is a collection of mathematical and statistical techniques used for optimizing complex processes that are influenced by different variables and their interactions. The aim is to simultaneously optimize the levels of the analyzed variables to achieve the best system performance while reducing the number of experimental trials. RSM has been successfully used for improving and optimizing extraction processes (Karabegović et al., 2013).

Therefore, the main goals of this study were to optimize the microwave-assisted extraction of antioxidants from tomato seeds by using the RSM. The effect of three independent variables namely microwave power, irradiation time and ethanol fraction on the extraction were studied. The comparison between the conventional extraction and MAE was also investigated.

Theoretical part

Chapter I

Tomato description and its by-products

1. Origin and history

The tomato belongs to the Solaneceae family, commonly known as a tomato plant. All related wild species of tomato are native to the Andean region that includes parts of Chile, Colombia, Ecuador, Bolivia and Peru. The most likely ancestor is the wild ***L.esculentumvar cerasiforme*** (cherry tomato).Which is indigenous throughout tropical and subtropical America. Although the ancestral forms of tomato grew in the Peru-Ecuador area, the first extensive domestication seems to have occurred in Mexico (Heuvelink, 2005).

2. Description

Tomato (***Solanumly copersicum)*** is an annual plant, which can reach a height of over two meters, The first harvest is possible 45-55 days after flowering, or 90-120 days after sowing .Tomato is a warm season crop , show a wide range of climatic tolerance, can be grown in the field wherever there are more than 3 months of frost- free weather. The shape of the fruit differs per cultivar. The color ranges from yellow to red (Naika et al., 2005; Zaghdani, 2002).

- **Botanical description of tomato plant**

Root: Vigorous tap root system that grows to a depth of 50 cm or more. The main root produces dense lateral and adventitious roots.

Stem: Growth habit ranges between erect and prostrate. It grows to a height of 2-4 m. The stem is solid, coarse, hairy and glandular.

Leaf: Spirally arranged, 15-50 cm long and 10-30 cm wide. Leaf is ovale to oblong, covered with glandular hairs. Inflorescence is clustered and produces 6-12 flowers. Petiole is 3-6 cm.

Flower: Bisexual, regular and 1.5-2 cm in diameter. They grow opposite or between leaves. Calyx tube is short and hairy, sepals are persistent. Usually 6 petals up to 1 cm in length yellow and reflexed when mature. 6 stamens, anthers are bright yellow in colour surrounding the style with an elongated sterile tip. Ovary is superior and with 2-9 compartments. Mostly self- but partly also cross pollinated. Bees and bumblebees are the most important pollinators.

Fruit: Fleshy berry, globular to oblate in shape and 2-15 cm in diameter. The immature fruit is green and hairy. Ripe fruits range from yellow, orange to red. It is usually round, smooth or furrowed.

Seeds: Numerous, kidney or pear shaped. They are hairy, light brown 3-5 mm long and 2-4 mm wide. The embryo is coiled up in the endosperm. Approximate weight of 1000 seeds is 2.5 – 3.5 g (Naika et al., 2005).

3. Morphology of the tomato fruit

Tomato fruit is attached to the rest of the plant by the pedicel at the receptacle, where sepals remaining from the flower structure are also attached (Figure 1.A). The pericarp tissue includes the outer pericarp, septa known also as radial pericarp, which separate the locules and the inner pericarp (Figure 1.B)(Jasionowicz., 2012).

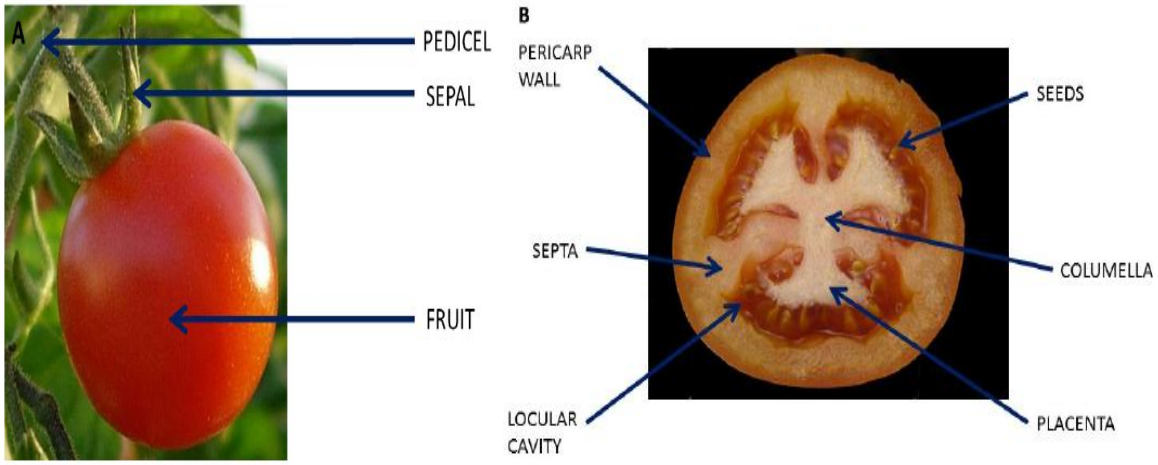


Figure 1: Morphology of tomato fruit. (A). Longitudinal-section through tomato fruit (B).

4. Economic importance and nutritional value of tomato

Tomato is known as healthy food, because of its special nutritive value and because of its wide spread production. It is considered as important commercial and dietary vegetable crop. Tomato is widely used fresh, as well as in processed and preserved products, like ketch-up, sauce, chutney, soup, paste, purée. Tomato is a rich source of minerals, vitamins and organic acids, essential amino acids and dietary fibers. It is also a rich source of vitamin A and C; it also contains minerals like iron, phosphorus**.** Nutritional value of tomatoes is presented in table I(Jasionowicz., 2012; Sainju et al., 2003).

Table I : Chemical composition of tomato fruit (figures for a small tomato of 100g) (Razdan and Mattoo, 2006)

Nutrient	Value
Moisture	95%
Food energy	22Kcal
Protein	1g
Fats	0.2g
Carbohydrates	4.7g
Fiber	0.5g
Clcium	13.0g
Phosphorus	27.0mg
Sodium	3.0mg
Magnesium	17.7mg
Potassium	244.0mg
Iron	0.50mg
Zinc	0.20mg
Copper	0.01mg
Vitamin A	900.0IU
Vitamin D	0
Vitamin E(α-Tocvopherol)	0.40mg
Vitamin C	23mg
Thiamin	0.06mg
Riboflavin	0.04mg
Niacin	0.70mg
Panthothenic Acid	0.33mg
Vitamin B-6(pyridoxine)	0.10mg
Folacin(folic acid)	39.00mg
Biotin	4.00mg
Vitamin B-12	0

Tomato is one of the most cultivated vegetables in the world, with 162 million tons produced in 2012. Details of tomato production per country are presented in table II(Jiang and Hsieh, 2015).

Table II: Tomato production around the globe (2012) (Messak,2014)

N°	Country	Production (tonne)
1	China	50125055
2	India	17500000
3	U.S.A	13206950
4	Turkey	11350000
5	Egypt Iran	8625219
6	Iran	6000000
7	Italy	5131977
8	Spain	4007000
9	Brazil	3873985
10	Mexico	3433567
11	Uzbekistan	2650000
12	Russian F	2456100
13	Ukraine	2274100
14	Nigeria	1560000
15	portugal	1392700
16	Morocco	1219071
17	Iraq	1100000
18	Tunisia	1100000
19	Greece	979600
20	Indonesia	887556
21	Cameroon	880000
22	Netherlands	805000
23	Algeria	796963
24	Syria	783874

5. Health effect of tomato

Several epidemiological studies suggest that consumption of tomatoes and tomato-based products reduces the risk of chronic diseases such as cardiovascular disease and cancer. In particular, intake of tomato and tomato based products has been relatively consistently associated with a lower risk of cancers of the prostate, lung and stomach. Recent intervention studies demonstrate that regular intake of small amounts of tomato products can increase cell protection from DNA damage induced by oxidant species. This protective action is typically attributed to antioxidant components like lycopene and other carotenoids, ascorbic acid, flavonoids and vitamin E (Raffo et al., 2006).

Lycopene has been shown to have strong antioxidant activity; it exhibits the highest physical quenching rate constant with singlet oxygen; it induces cell-to-cell communication; and it modulates hormones, immune systems, and other metabolic. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, cardioprotective, and vasodilator effects (Navarro-González et al., 2011).

6. By-products from tomatoes

Food industries generate large amounts of wastes and by products that contain highly valuable biologically active compounds. The amount of tomato processing wastes approximately are around 37,000–39,000 tonnes/year in Hungary. This waste contains the carotenoid rich skin and the seeds of tomato. As it is available in high amount it can be an economic and reasonable choice to produce health promoting products rich in lycopene, carotenoids and/or natural oil (Vági et al., 2007).

The manufacture of tomato juice and puree provides peels and seeds as residue. They account for about 4.5% of the fresh weight: 3% peels and 1.5% seeds. In some countries, seeds are used for oil production. Following seed pressing or extraction for oil production 73-82% tomato seed oil meal is left as residue. The production of peeled tomatoes provides only peels as residue. Tomato pomace (TP) is a by-product from the processing of tomato paste. After juice is extracted, a residue, wet TP, which is primarily consisting of water, tomato seeds and peels, is left. The high water content (75%) of this byproduct limits its length of storage. Because of storage problems, wet TP is often dried. However, artificial drying increases the price of TP substantially; hence, much of the TP

now produced is discarded or fresh fed only limited periods of time. Wet TP would spoil in two days if exposed to the air. Tomato pomace can be fed to ruminant animals for longer periods of time without spoilage, when it is ensiled with or without additives. Addition of grain and straw to TP during ensiling can lower dry matter content of pomace and also eliminate excess water drainage and nutrition losses. Tomato processing by-products can be a valuable energy and nutrient source obtained more cheaply than alternative ingredients to feed ruminants, when it is appropriately preserved (Mirzaei-Aghsaghali and Maheri-Sis, 2008).

7. Phenolic compounds of tomatoes seed

By-products derived from the tomato industry have a potential application in the food industry; due to their content of bioactive compounds, but they are also attractive for the pharmaceutical or cosmetic industries**.** The peels and seeds of tomatoes have been found to contain more phenolic compounds than the fleshy pulp in 12 genotypes of tomatoes. The free phenolic content (expressed as mg catechin/100 g, fresh weight) in pulps is ranged from 9.2 to 27.0 mg/100 g, compared to 10.4 to 40.0 mg/100 g in peels, and also that for each genotype, the phenolic content in peel was higher than in pulp. The total phenolics content (expressed as mg gallic acid equivalents/100 g) of skin and seeds in tomatoes were, respectively, 29.1 and 22.0, compared to 12.7 mg /100 g in the pulp (table III) (Balasundram et al., 2006; Navarro-González et al., 2011).

Phenolic compounds in tomatoes are mainly represented by flavanones (naringeninglycosilated derivatives) and flavonols (quercetin, rutin and kaempferolglycosilated derivatives) (Vallverdú-Queralt et al., 2012)**.**

Table III: Major antioxidants and antioxidant activities in the skin, pulp and seed fractions (means ± standard error of means of the three cultivars) (Toor and Savage, 2005).

	Total phenolics (mg GAE/100 g)		**Total flavonoids (mg /100g)**	**Lycopene (mg/100g)**	**Ascorbic acid(mg/ 100g)**	**Antioxidant activity (µMTEAC/100g)**	
Fraction	**hydrophilic**	**lipophilic**				**hydrophilic**	**lipophilic**
Skin	29.1±1.12	5.6±0.21	20.4±0.61	8.7±1.12	16.9±0.89	212.6±14.83	18.5±0/72
Pulp	12.7±2.03	2.3±0.12	8.2±0.37	2.8±0.37	8.9±0.59	81.8±9.49	7.0±0.35
Seeds	22.0±3.76	3.5±0.33	12.1±1.18	1.6±0.10	8.4±0.78	114.0±20.12	9.4±1.16

Chapter II

Microwave assisted extraction

1. History

A reliable device for generating fixed frequency microwaves was developed at the University of Birmingham a part of RADAR development during World War II. In the 1950s, domestic and commercial applications of microwave in heating and cooking appeared in the US. The application of microwave in chemistry is a result of the wide spread of domestic microwave oven in the 1970s .Samra *et al*. had first time used microwave domestic ovens for the treatment of biological samples for metal analysis in 1975. The application of MAE for plant materials was first reported by Ganzler and co-workers in 1986 (Dai, 2006; Ankit , G et al., 2012)**.**

2. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is a relatively new extraction technique, which utilizes microwave energy to heat the solvent and the sample and to increase the mass transfer rate of the solutes from the sample matrix into the solvent. The usage of microwaves for extracting plant constituents is still in infancy(Ajay Shukla et al., 2013).

Microwaves lie between infrared radiation and radiowaves in the region of the electromagnetic spectrum (fig 3)**.** It is an electromagnetic radiation with a wavelength from 0.001 m to 1 m (i.e. with a frequency from 3×10^{11} Hz to 3×10^{8} Hz), which can be transmitted as the wave. This range is most used as the frequency for communications, in particular the radar, cell phones, television and satellite applications, therefore the Federal Communications Commission agreement states that two frequencies of 0.915 and 2.45GHz are specifically used for microwave heating in order to avoid interference with communications. which correspond to wavelengths of 33.5 and 12.2 cm, respectively (Al-Harahsheh and Kingman, 2004; Jang et al., 2009;Menéndez et al., 2010; Zhang et al., 2011;).

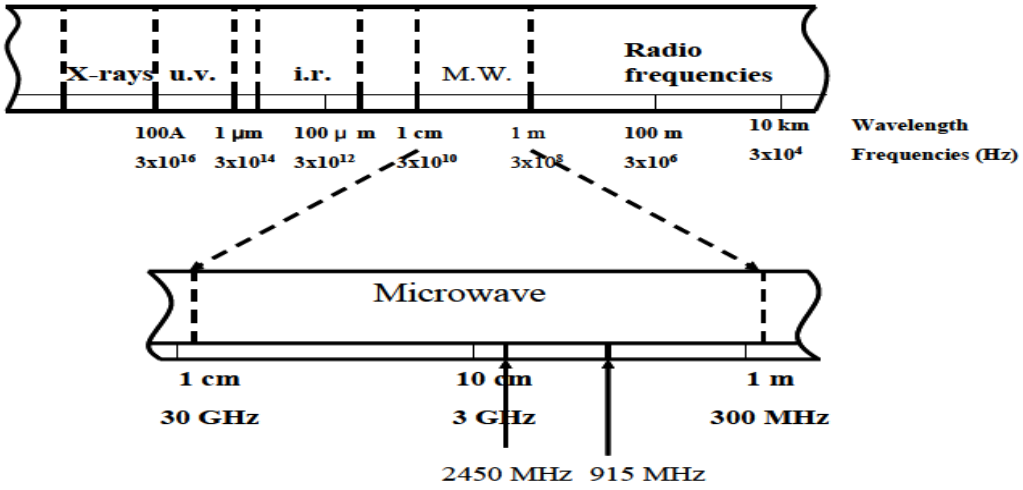


Figure 2 : Locations of microwaves on the electromagnetic spectrum (Dai, 2006).

3. Principle

Microwave assisted extraction consists of heating the solvent in contact with the sample by means of microwave energy. The process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of the ions, which enhance penetration of the solvent into the matrix, allowing dissolution of the components to be extracted (Jain et al., 2009).

Microwave heating is based dielectric heating, the ability of some polar liquids and solids to absorb and convert microwave energy into heat. In this context, a significant property is the mobility of the dipoles by either ionic conduction or dipolar polarization and the ability to orient them according to the direction of the electric field. 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 and Rafiee, 2011) .

4. Factors influencing the performance of MAE

There are a number of factors that one must consider when using microwave assisted extraction method such as the choice of solvent, microwave application time, microwave-power.

4.1. Choice of solvent:

A correct choice of solvent is fundamental for obtaining an optimal extraction process. When selecting solvent, consideration should be given to the microwave-absorbing properties of the solvent, the interaction of the solvent with the matrix, and the analyte solubility in the solvent. If the solvent molecule is not able to absorb microwave energy there will be no heating and hence no effective extraction (Eskilsson and Björklund, 2000).

4.2. Microwave power

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 (Chan et al., 2011).

4.3.Extraction time

Time period of heating is another important factor that influences the extraction process of MAE. The quantity of analyte extracted can be increased with an increase in the extraction time, but there is an associated risk of degradation of thermo labile components. Varying time periods are required for extraction of different matrices, but exposure of even few seconds have demonstrated to give excellent yields (Afoakwah et al., 2012).

4.4. Solvent volume

The amount of solvent needed for a single sample is often in the range of 10–30 ml. In some cases solvent volume may be an important parameter for efficient extractions. The solvent volume must be sufficient to ensure that the entire sample is immersed, especially when having a matrix that will swell during the extraction process (Eskilsson and Björklund, 2000).

5.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. Most of the commercial microwave systems are equipped with temperature control with monitoring system and pressure control(Chan et al., 2011).

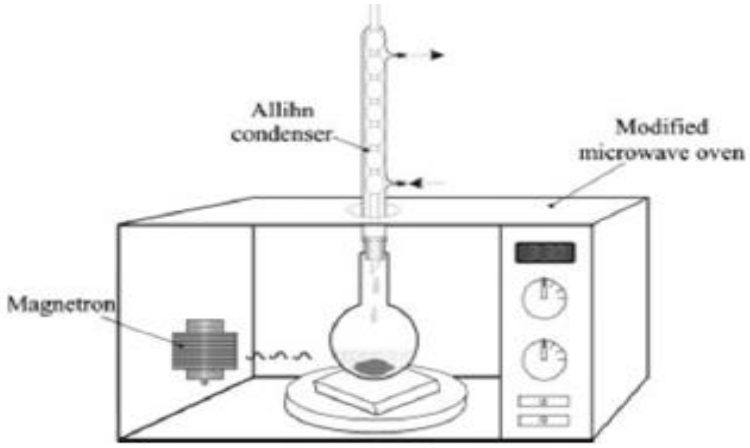


Figure 3: Microwave equipment

6. Advantages and disadvantages

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 extraction , microwave assisted extraction generally shows evident advantages with shorter extraction time, higher extraction yield, higher selectivity and better quality of the target extracts. By considering economical and practical aspects, MAE is a strong novel extraction technique for the extraction of nutraceuticals.

Microwave irradiation can accelerate the chemical reactions or changes of some target secondary metabolites, and other operational conditions (e.g., high extraction pressure) of microwave assisted extraction may modify the chemical structures of the target compounds, both of which might result in the reduction of extraction yield. Furthermore, the efficiency of microwaves can be very poor when either the target compounds or the solvents are non-polar, or when they are volatile (Zhang et al., 2011; Wang and Weller, 2006).

Chapter III

Response surface methodology

1. General information

Response surface methodology (RSM), originally described by Box and Wilson (1951) , enables evaluation of the effects of several process variables and their interactions on response variables. Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes, in which a response of interest is influenced by several factors (independent variables). RSM not only defines the effects of the independent variables, but also generates a mathematical model, which describes the chemical or biochemical processes. Currently, RSM is being used in the extraction of phenolic compounds from various sources (Liu et al., 2010; Liyanapathirana and Shahidi, 2005 ;Radojkovica et al., 2012).

2. Terminology

2.1. 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 et al., 2008).

2.2. Experimental design

Experimental design is a specific set of experiments defined by a matrix composed by the different level combinations of the variables studied (Bezerra et al., 2008).

2.3. Factors or independent variables

Factors or independent variables are experimental variables that can be changed independently of each other. Typical independent variables comprise the pH, temperature, reagents concentration, microwave irradiation time, flow rate, atomization temperature, and elution strength, among others.(Bezerra et al., 2008).

2.4. Responses or dependent variables

Responses or dependent variables are the measured values of the results 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 et al., 2008).

2.5. Coded factor levels

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 (Candioti et al., 2014).

2.6. 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. 4 a) shows the is response lines as a function of the levels of two factors, while a 3D response surface plot (Fig. 4b) 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. 4 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 and Heyden, 2011) .

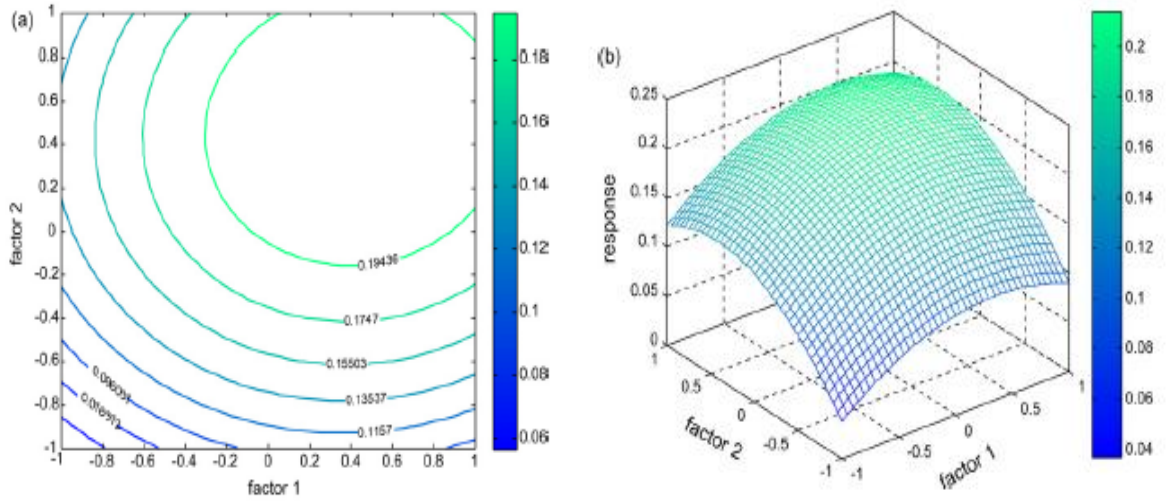


Figure 4: (a) 2D contour plot, and (b) 3D response surface plot.

3. Steps for RSM application

The optimization by means of RSM approach could be divided into six stages (Fig 5): (1) selection of independent variables and possible responses, (2) selection of experimental design strategy, (3) execution of experiments and obtaining results, (4) fitting the model equation to experimental data, (5) obtaining response graphs and verification of the model (ANOVA), (6) determination of optimal conditions (Witek-Krowiak et al., 2014).

4. Advantages and disadvantages of RSM

RSM has several advantages compared to the classical experimental or optimization methods in which one variable at a time technique is used.

- Firstly, RSM offers a large amount of information from a small number of experiments. Indeed, classical methods are time consuming and a large number of experiments are needed to explain the behavior of a system.
- Secondly, in RSM it is possible to observe the interaction effect of the independent parameters on the response. Especially in biochemical processes, the interaction effect of the parameters would be more critical such as synergism, antagonism, and addition (RSM is a useful tool for the optimization of chemical and biochemical process).

On the other hand, the major drawback of RSM is to fit the data to a second order polynomial. We cannot say that all systems containing curvature are well accommodated by the second order polynomial (Radojkovića et al., 2012).

The experimental part

Chapter IV

Materials and methods

1. Reagents

The compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate, Gallic acid and Folin–Ciocalteu phenol reagent were purchased from Sigma–Aldrich (Germany).All solvents used were of analytical grade and purchased from Prolabo (CE).

2. Plant material

Tomato seeds were collected from Fouara factory in Setif. The tomato seeds are by products of tomato paste. The samples were dried for about 1 day in a stove at 40C°. Dried by-products were ground with an electrical grinder (IKAmodel A11Basic.Germany) and the powder obtained was passed through 500 µm sieve.

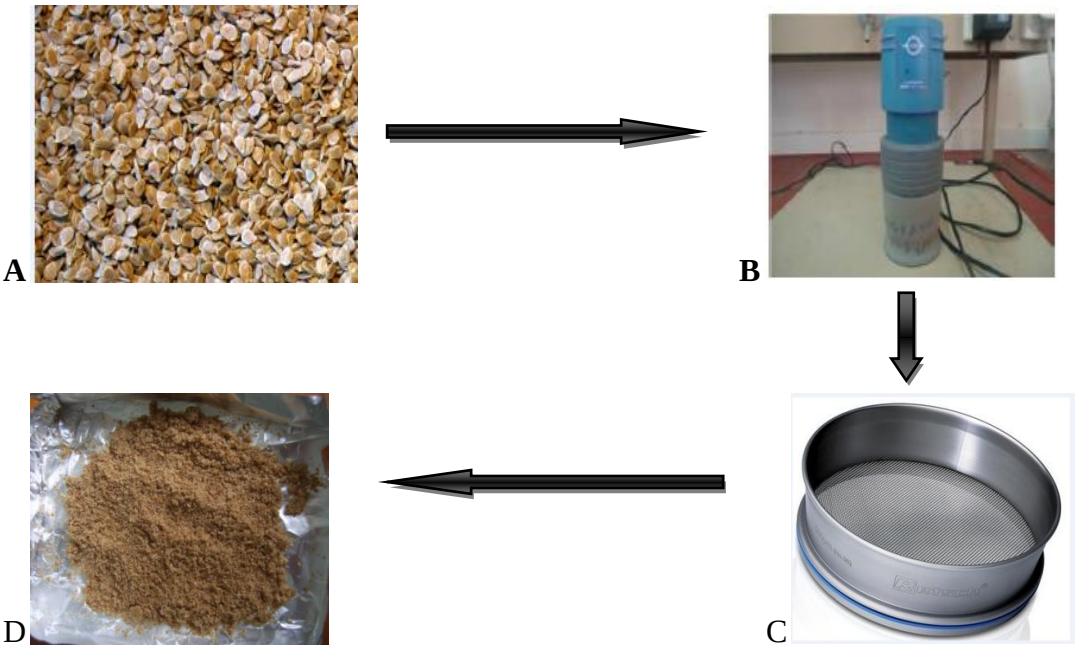


Figure 5: **A**: tomatoes seeds; **B**: Electrical grinder, **C**: sieve, **D**: tomato powder seed

3. Activity of water

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

Water activity of the powder of the tomato seeds was 0.554 at 25°C.

Figure 6: WA measures

4. Experimental work

For optimization of the MAE procedure, the influences of the process parameters were firstly separately investigated in single-factor experiments to limit the total experimental work (Tables II). When one variable was not studied, it was kept constant. Response surface methodology (RSM) was used to determine the optimal conditions for extraction. Central composite design (CCD) was used to investigate the effects of three independent variables; microwave power, irradiation time and solvent fraction at three levels on the dependent variables TPC yield and antioxidant activity. CCD uses the method of least squares regression to fit the data to a quadratic model.

The quadratic model for each response was as follows:

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum\sum \beta_{ij} X_i X_j$$

Where Y is the predicted response, β_0 a constant, β_i the linear coefficient, βii the quadratic coefficient, βij the interaction coefficient of variables i and j, and Xi and Xj are independent variables. The software uses this quadratic model to build response surfaces. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA) that was generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level (p = 0.05). Three-dimensional response surface plots were generated by keeping one independent variable at its zero level and plotting this against two factors (independent variables). The codes used in the response surface analysis and the corresponding parameter values are given in table III.

To verify the adequacy of the models, additional extraction trials 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. Efficiency of the MAE was compared to conventional extraction methods based on the TPC and antioxidant activity (according to DPPH assay) which were measured only on the extracts obtained under the optimum conditions selected by RSM.

5. Extraction procedures

5.1. Microwave assisted extraction

A domestic microwave oven (NN-S674MF.Samsung. Malaysia) with cavity dimensions of 22.5 cm × 37.5 cm × 38.6 cm and 2450 kHz working frequency was used. The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter linearly adjustable from 100 to 900 W). The oven was modified in order to condensate into the sample the vapors generated during extraction giving a constant sample volume. Schematic diagram of microwave equipment was shown in Figure 7.

For the extraction, 1 g of the sample was placed in a round-bottom flask with 30 ml of solvent. The flask was placed in a microwave oven and connected to condenser. After irradiation. The flask was taken out and the solution was filtered through filter paper.

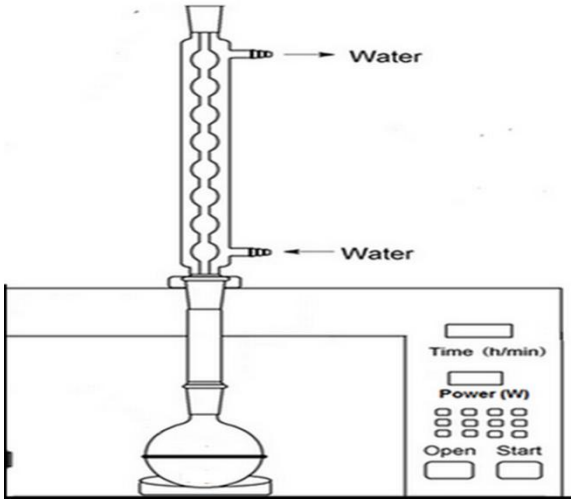


Figure 7: Schematic diagram of microwave equipment

5.2. Conventional extractions

Extraction by maceration and stirring were done in order to compare with MAE. 1 g of sample was extracted with solvent for 30, 60, 120 min in the Erlenmeyer by agitation (with the help of magnetic stirrer) and for 1,6, 24 hours by maceration. After each extraction, the mixture was filtered through filter paper

6. Total phenolic compouds

The content of TPC was determined by the Folin–Ciocalteu method (figure 8). The volume of 1millilitre of Folin–Ciocalteu reagent (diluted ten times by water) was mixed with 200µL of the extract. After 5min, 1 ml of aqueous solution of sodium carbonate (6%) was added. The mixture was kept for 30 min at room temperature. Absorbance was measured at 750nm. Ethanol solution of gallic acid was used as standard (Dahmoune et al., 2013).

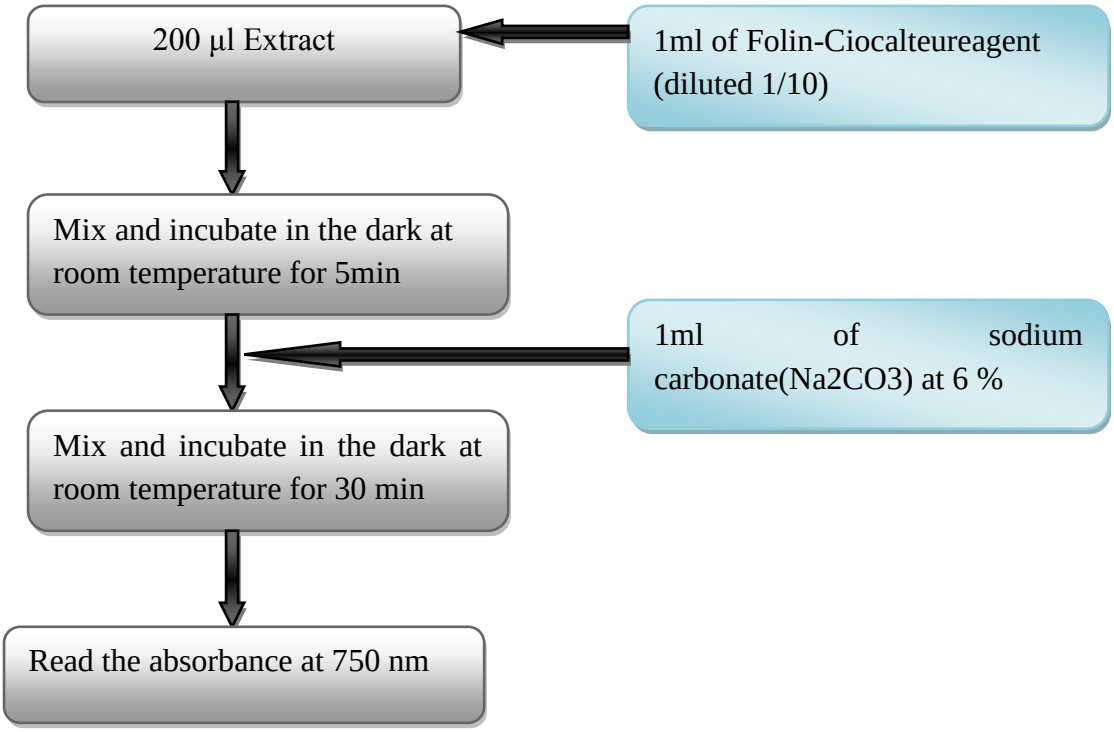


Figure 8: Analytical protocol used for determination of TPC

7. Antioxidant activity

The antioxidant activity of the extracts was estimated by the DPPH method, according to the procedure described by Haddadi-Guemghar et al. (2014) (figure 9). An aliquot of 0.5 ml of sample solution was mixed with 1.5 ml of ethanolic solution of DPPH (0.2 mM).

The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 515nm with a spectrophotometer. Ethanol instead of sample solution was used as a control. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and was calculated using the following equation:

$$Scavenging \quad activity(\%) \quad = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c and A_s are the absorbance at 515nm of the control and sample, respectively.

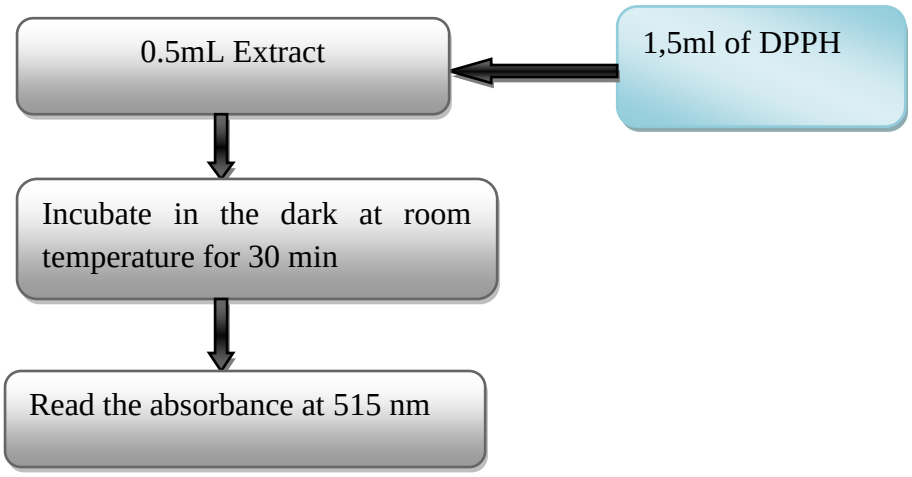


Figure 9: Analytical protocol used for determination of antioxidant activity

8. Statistical analysis

Each extraction trial and all the analyses were carried out in triplicate and all the data in this work have been reported as means ± S.D. Influence of each factor on the TPC yield, and antioxidant activity in the single-factor experiment for the MAE was statistically assessed by ANOVA and Tukey's post hoc test with 95% confidence level. RSM was performed using the Design Expert software (Version 8.0.1. Stat-Ease, Inc. Minneapolis, MN program).

Chapter V

Results and discussion

1. Preliminary study for selection of appropriate extraction conditions

In this study, the effects of several influential extraction parameters; microwave power, irradiation time, solvent type, solvent-to-solid ratio were systematically studied separately in single factor for set-up of the optimal extraction conditions to obtain the maximum TPC yield from tomato seeds. Preliminary study was performed in order to determine the appropriate experimental ranges to be considered during the optimization process. The results were shown in table IV.

Table IV: Results of single-factor experiments for microwave assisted extraction. Results are reported as means ± S.D. Same letters in the same column refers to means not statistically Different according to ANOVA and Tukey's test. TPC, total phenols yield referred to dry weight (dw) of tomato seeds; GAE, gallic acid equivalents.

Solvent		**Microwave power**		**Irradiation time**		**Solvent fraction**	
Type	TPC yield (mgGAE/100g)	(W)	TPC yield (mgGAE/100g)	(s)	TPC yield (mgGAE/100g)	(mL)	TPC yield (mgGAE/100g)
80% Methanol	202.87±5.64^{b}	200	187.13±1.36^{d}	20	230.41±15.17^{b}	20	223.61±15.72^{c}
80% Ethanol	242.16±10.89^{a}	400	206.34±2.37^{c}	40	226.21±27.38^{b}	30	354.27±32.42^{a}
80% Acetone	173.69±7.54^{c}	600	238.24±3.58^{b}	60	260.75±6.37^{a}	40	333.66±5.15^{a}
		800	266.11±6.72^{a}	80	186.55±9.93^{c}	50	280.70±2.47^{b}
				100	226.54±19.35^{b}		
				120	268.08±9.7^{a}		

1.1.Effect of solvent type

Solvent choice for MAE is dictated by the solubility of the extracts of interest, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent determined by its dielectric constant**.** The effectiveness of MAE will largely depend on the capacity of the extracting solvent for absorbing and transmitting the microwave (Mandal and Mandal, 2010; Wang and Weller, 2006)**.**

Different type of solvents (80%methanol, 80%ethanol and 80% acetone (v/v)) were used to investigate the influence of solvent on the recovery of TPC when the other extraction conditions were set as follows: microwave power 500W, extraction time 60 s, ratio of liquid to solid 20 mL/g(table IV).Type of solvent significantly influenced the TPC

yield, with aqueous ethanol being the best one. Aqueous ethanol was then selected as the solvent for the RSM trials.

1.2. Effect of microwave power

Different microwave power was set at 200, 400, 600 and 800Wto investigate the influence of microwave power on the recovery of TPC when the other extraction conditions were set as follows: extraction time 60 s, ethanol proportion 80%, ratio of liquid to solid 20 mL/g. Table IV shows that the extraction of TPC was greatly influenced by the microwave power. The extraction increased with the increase of microwave power. The range 300–900 W was selected for the RSM study, while the 800 W powers was used for the last single-factor trials.

1.3. Effect of irradiation time

Irradiation time is another important factor that influences the extraction process of MAE. The quantity of analyte extracted can be increased with an increase in the extraction time, but there is an associated risk of degradation of thermo labile components (Afoakwah et al., 2012).

The recovery of TPC affected by different extraction time (20, 40, 60,80, 100, and 120 s) is shown in table IV, when other three factors(microwave power, ethanol proportion and ratio of liquid to solid) were fixed at 800 W, 50% (v/v) and 20 mL/g. The results indicate that the recovery of TPC increased with the increase of MAE time. The recovery could reach its maximum after 60s and after 120s of MAE. Based on these results, 60 s was selected for the next single-factor trials, while the range 20–100s was selected for the RSM trials.

1.4. Effect of solvent-to-solid ratio

The solvent volume always must be sufficient to ensure that the entire sample is immersed during the extraction process. Generally in conventional extraction techniques a higher volume of solvent will increase the extraction performance, but in MAE a higher solvent volume may give lower yield(Mandal and Mandal, 2010).

The recovery of TPC affected by different ratio of liquid to solid (20, 30, 40, and 50 mL/g) can be seen in table IV, when the other three factors (microwave power, extraction time, and ethanol proportion)were fixed at 800 W, 60 s, and 80% (v/v). The result implied the recovery of TPC was enhanced to the critical value (354.51±0.27

mg/mL) at the ratio of 30mL/g. Then, the ratio 30 mL/g was selected for the RSM optimization.

2. Optimization of microwave assisted extraction procedure

The experimental domain was defined taking into account preliminary experiments. Each factor was evaluated at three levels at the following intervals: 300-900 W for microwave power, 20–100s for extraction time, 0–80% for ethanol fraction. However liquid to solid ratio were fixed in all experiments at 30mL/g. The three factors and lower, middle and upper design points for RSM in coded and natural/ un coded values are shown in Table III. In RSM, natural variables are transformed into coded variables that have been defined as dimensionless with a mean zero and the same spread.

Table V: The coded values and corresponding actual values of the optimization parameters used in the response surface analysis.

Code	Microwave power (W)	Irradiation time (s)	Ethanol fraction (%)
-1	300	20	0
0	600	60	40
1	900	100	80

The experimental design matrix is shown in table VI. The extraction efficiency of the microwave process was estimated by measuring TPC and the antioxidant activity of tomato seeds extracts. Two second order polynomial models were generated to describe the empirical relationship between the TPC and the antioxidant activity with operational conditions (microwave power A, irradiation time B and ethanol fraction C).

TPC=+263.86+4.85A+28.22B-21.77C-2.59AB+8.21AC+3.76BC-54.72A^2-31.56B^2-37.08C^2 (1)

Antioxidant activity =+65.10+2.80A+6.58B-0.51C+2.37AB-2.38AC+3.14BC+2.85A^2 7.07B^2+9.44C^2 (2)

Table VI: Three-factor, three levels central composite design used for RSM and experimental data of the investigated responses of tomato seeds extracts.

Run	Factor A Microwave Extraction (w)	Factor B Irradiation time(s)	Factor C Ethanol Fraction(%)	Response 1 TPC (mgGAE/100g)	Responce 2 antioxidant Activity (%)
1	600	60	40	290	66.9045
2	900	20	80	103.37	57.3108
3	900	100	0	174.94	81.3711
4	600	60	0	247.86	73.7063
5	600	60	40	280.138	67.7674
6	300	20	80	82.6017	63.199
7	600	60	40	251.945	62.2346
8	300	100	80	137.257	76.2951
9	600	60	40	294.311	64.671
10	600	20	40	189.752	50.8339
11	600	100	40	252.257	62.326
12	300	60	40	197.827	63.8081
13	600	60	80	183.107	72.4881
14	900	20	0	145.666	71.3714
15	600	60	40	275.794	66.6507
16	600	60	40	236.182	68.1227
17	900	60	40	197.848	69.1938
18	300	100	0	197.386	68.2546
19	900	100	80	173.612	83.9598
20	300	20	0	131.816	63.6558

2.1. Fitting the Model

Table VII listed the analysis of variance (ANOVA) for the fitted quadratic polynomial models of TPC and antioxidant activity .F-test suggested that models had a very high model F-valueand a very low *p*-value ($p < .0001$), indicating these models were highly significant. The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As shown in TableV, *p*-value of the lack of fit of all responses were .superior to 0.05,which implied they were not significant relative to the pure error and indicated that the models equations were adequate for predicting the yield of TPC and antioxidant activity under any combination of values of the variables.

Table VII: ANOVA for the effect of microwave power, irradiation time and ethanol fraction on TPC and antioxidant activity using a quadratic response surface model.

source	TPC		antioxidant Activity	
	F value	P value	F value	P value
Model	17.46	<0.0001	21.81	<0.0001
A-Microwave power (W)	0.53	0.4837	14.51	0.0034
B-Irradiation time (s)	17.88	0.0017	80.25	<0.0001
C-Ethanol fraction (%)	10.64	0.0085	0.48	0.5030
AB	0.12	0.7359	8.31	0.0163
AC	1.21	0.2968	8.40	0.0159
BC	0.25	0.6256	14.64	0.0033
A^2	18.48	0.0016	4.13	0.0695
B^2	6.15	0.0326	25.47	0.0005
C^2	8.49	0.0155	45.42	< 0.0001
Lack of fit	0.72	0.6346	1.18	0.4308

To evaluate the goodness of the fit of the models, different descriptive statistical analysis such as determination coefficient (R^2), adjusted determination coefficient ($AdjR^2$), prediction determination coefficient (Pred. R^2), adequate precision (Adeq Precision) and coefficient of variance (CV) were used (Table VIII). The R^2 values for TPC and antioxidant activity were 0.9402 and 0.9515 respectively, which implied that the sample variation was statistically significant at 94.02% and 95.15% for TPC and antioxidant activity and only 5% (aproximatively) of the total variance could not be explained by the models. In others terms determination coefficient close to 1indicates a high degree of correlation between the observed and predicted data. The Adj R^2 and the Pred R^2 of both TPC and antioxidant activity were also satisfactory to confirm the significance of the models. The Pred. R^2were in reasonable agreement with the Adj. R^2; the difference between the two parameters is less than 0.20 in both TPC and antioxidant activity models.

Coefficient of variation (CV) describes the extent to which the data were dispersed. In general, a small value of CV gives a better reproducibility, and a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model. As a general rule, the CV should not be greater than 10%**.** The CV for TPC and antioxidant activity was within the acceptable range(Liyanapathirana and Shahidi, 2005 ;Prakash Maran and Manikandan, 2012) .

Table VIII: Descriptive parameters of the model fitting.

	TPC model	**Antioxidant activity model**
R^2	0.94	0.95
Adj.R^2	0.8864	0.9079
Pred.R^2	0.7463	0.7129
CV	10.44	3.43

2.2. Significance of the variables

The significance of each coefficient was determined using *p-value* in table VII. The *p*-value is used as a tool to check the significance of each coefficient and the interaction strength between each independent variable. The *p* value less than 0.05 indicate model terms are significant. In this case irradiation time and quadratic terms of microwave power

were the major factors affecting the extraction of TPC followed by ethanol fraction and then by quadratic terms of ethanol fraction and irradiation time.

Irradiation time and quadratic term of ethanol fraction were the most factors affecting the antioxidant activity. However it can be seen that ethanol fraction(C) did not influence the antioxidant activity but its interaction with others factors and its quadratic terms did it.

After neglecting all the non significant terms (p>0.05), the fitted quadratic models for TPC and DPPH in coded variables are given in Eqs (3) and (4), respectively.

TPC=+263.86+28.22B-21.77C-54.72A^2-31.56B^2-37.08C^2 (3)

AA= +65.10+2.80A+6.58B+2.37AB-2.38AC+3.14BC+2.85A^2-7.07B^2+9.44C^2 (4)

2.3. Analysis of response surface

The 3D response surface is the graphical representation of regression equation. It provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables (Zhong and Wang, 2010).The effects of the independent variables and their mutual interaction on the extraction yield of TPC and antioxidant activity can be seen on three dimensional response surface curves shown in Fig 10. The plots were generated by plotting the response using the z-axis against two independent variables while keeping the other independent variable at zero level.

Fig 10 is a response surface plot showing the effect of microwave power and irradiation time on the TPC yield at the fixed ethanol fraction of 40%. The microwave power was shown as a negative quadratic effect on the yield (p<0.001). The yield of extraction increased up to about 600W followed by a decline with further increase of microwave power. The accelerated extraction of TPC using microwave power can be correlated with the direct effects of microwave energy on phytomolecules by ionic conduction and dipole rotation, which produces power, dissipated in a volumetric fashion inside the solvent and plant material that then generates molecular movement and heating. It is known that the temperature of the extraction medium increases with increased microwave power. These increased temperatures result in improved extraction efficiencies, as desorption of analytes from active sites in the matrix will increase, however very high temperatures may cause degradation of analytes. For a long extraction time, the negative quadratic effect was also significant (p<0.03). This was reflected in the plateau of the TPC yield for the extraction

time over 77s. A further increase in extraction time resulted in little change for the yield of TPC. The irradiation time was the most factor affecting the extaction of TPC from tomato seeds by microwaves. The increase of the irradiation time from 20s to 77s, over a microwave power of 600W, improved TPC yield up to 24%.

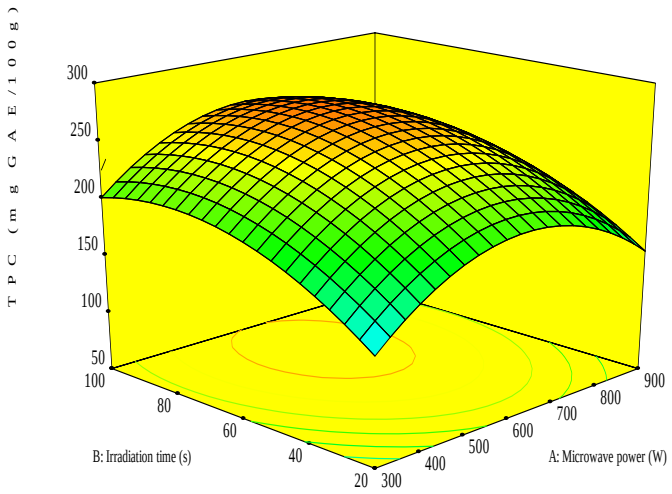


Figure 10: Surface plot of the TPC yield as a function of microwave power and irradiation time at ethanol fraction of 40%

The effect of polarity of solvent on yield of TPC is presented in Fig.11 The yield increased significantly with an increase in ethanol concentration up to 29% and then declined (Fig.11). The optimum was obtained with low concentration of ethanol. The negative effect of ethanol at high levels was probably due to the coagulation of proteins which make a resistance to phenolic compounds diffusion. Similar results was found by Yang et al. (2009). Who obtained a best extraction of crocin from Gardenia jasminoides Ellis fruits by low concentration of ethanol .

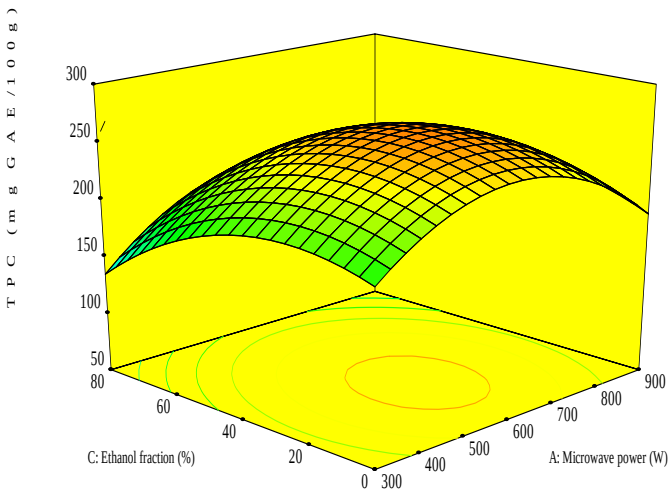


Figure 11: Surface plot of the TPC yield as a function of microwave power and ethanol fraction at irradiation time of 60s

Fig.12 is a response surface plot showing the effect of microwave power and ethanol fraction on the antioxidant activity at the fixed irradiation time of 60s. The microwave power was shown as a linear effect on the antioxidant activity ($p< 0.003$). The yield of extraction increased with the increasing of microwave power. Negative interaction was shown between microwave power and ethanol fraction which means that at the low microwave power the best extraction of antioxidants was obtained by 80% ethanol however at high microwave power the best extraction of antioxidants was obtained by water. This result suggests that the heat generated by microwaves increase the coagulation of proteins by high concentration of ethanol and so, we have obtained a best antioxidant activity with 80% ethanol at low microwave powers and a best antioxidant activity with water at high microwave powers.

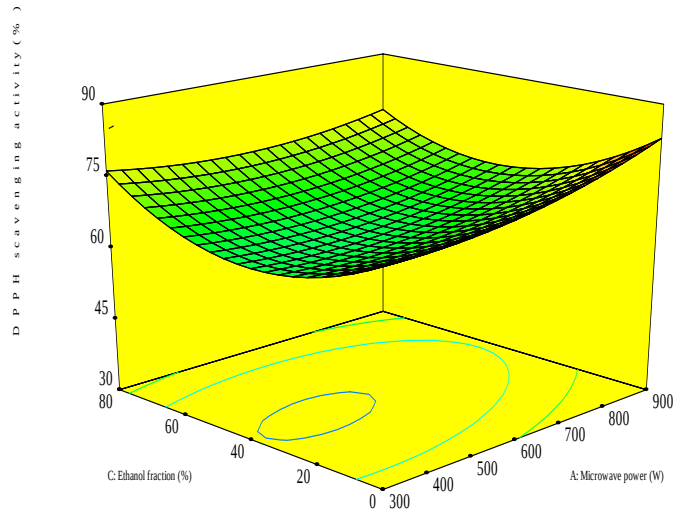


Figure 12: Surface plot of the antioxidant activity as a function of microwave power and ethanol fraction at irradiation time of 60s

The irradiation time exhibited significant linear and negative quadratic effects on the antioxidant activity (Table V and Fig. 13).The increase of the irradiation time from 20s to 76s, over a microwave power of 900W and water, improved antioxidant activity up to 14%.

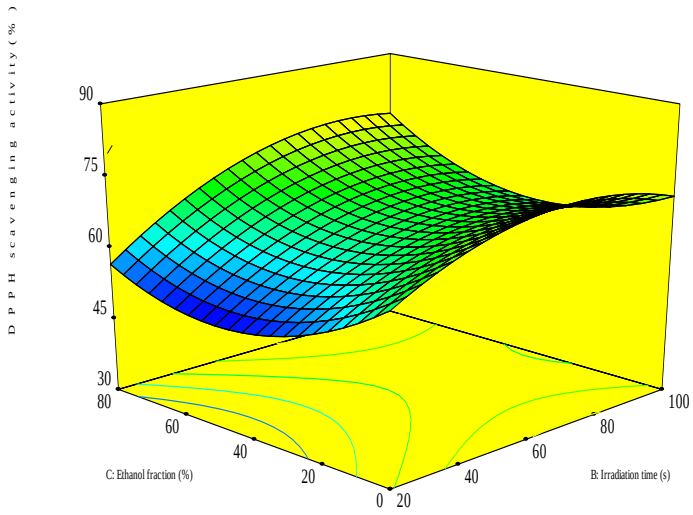


Figure 13: Surface plot of the antioxidant activity as a function of irradiation time and ethanol fraction at microwave power of 600W

3. Optimal extraction conditions

Using a quadratic model to describe the experimental we optimized three experimental variables for maximal extraction of phenolic compounds, and antioxidant activity from tomato seeds. The results of the optimization are summarized in Table IX. When comparing the optimal conditions based on TPC to those obtained for antioxidant activity, it was found that the optimums of irradiation time were closely related. However, there was a difference in the concentration of microwave power and ethanol required for optimal extraction of TPC and antioxidant activity.

Table IX: Optimum conditions for the MAE of TPC (mg EAG/100g DW) and antioxidant activity (%) from tomato seeds .[a]

	Microwave power (W)	**Irradiation time (s)**	**Ethanol fraction (%)**	**Prediction**	**Experimental**
TPC	600	77.22	29.18	272.91± 17.14	266.97±10.70
Antioxidant activity	900	76.42	0	84.27±4.26	82.30±4.88

[a] All values represent means±SD

Simultaneous optimization, using the Desirability function (Fig. 14) indicated that the optimum conditions for extraction of antioxidants and phenolics resulted in A = 745W, B = 74s, and C = $2.98e^{-14}$ ethanol, with a desirability value of 0.776. At this point, the investigated responses were theoretically calculated (with Eqs. 1 and 2) as TPC: 238.40±32.00 mg GAE/100 g DW and antioxidant activity: 78.96±3.52. These results evidence the advantage of applying simultaneous optimizations because when optimizing only TPC, a high value was found for this variable but the value obtained under these conditions for antioxidant activity was not as good as when optimizing only antioxidant activity. In the same way, when optimizing only antioxidant activity, a high value was found for this variable but the value obtained under these conditions for TPC was lower than that found in the previous analysis. In this regard, the simultaneous optimization achieves a compromise finding good values for both variables that are being optimized.

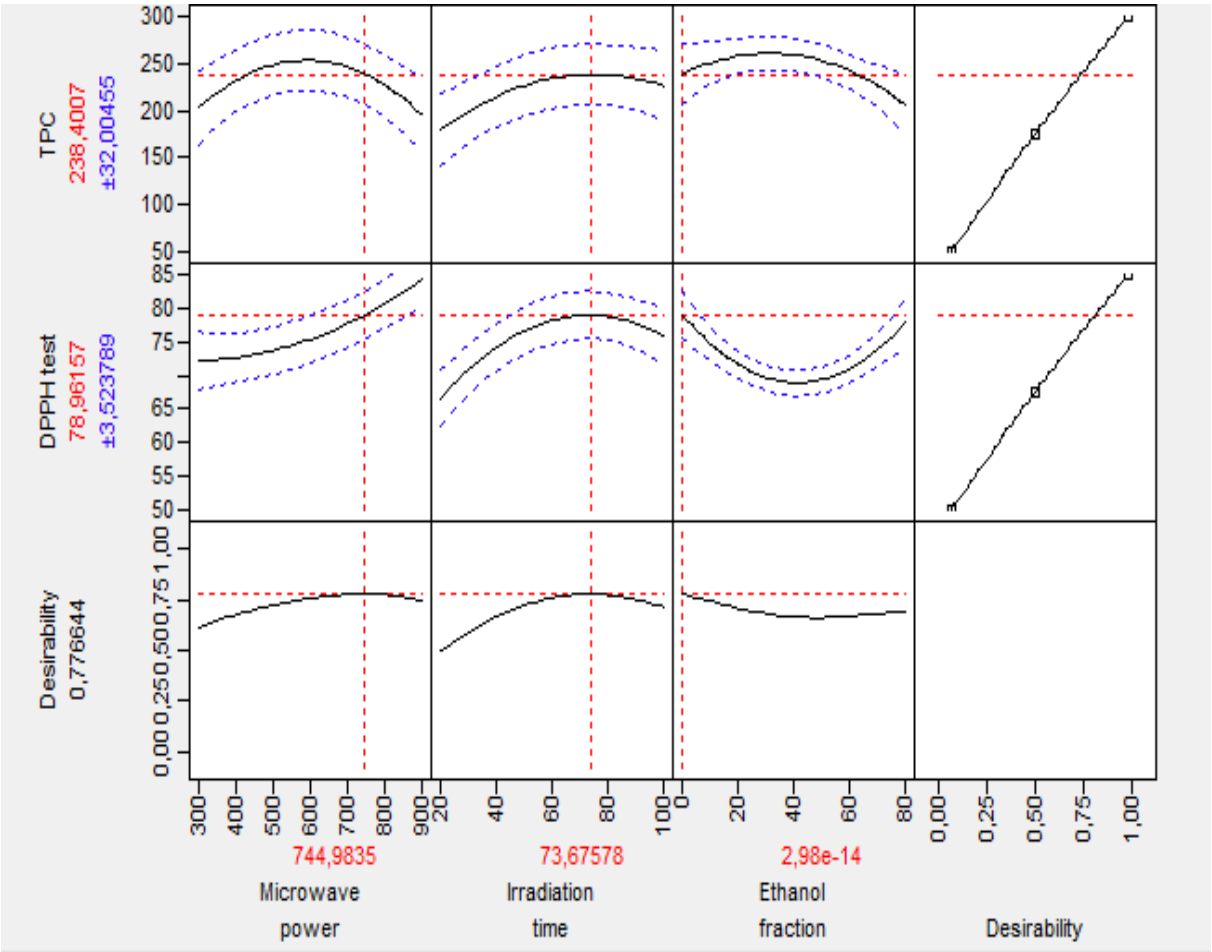


Figure 14: Profiles for predicted values and Desirability function.

4. Validation of the models

The optimized conditions obtained by RSM were used to validate the predictive model of extraction for TPC, and antioxidant activity from tomato seeds. Table IX shows that experimental values are reasonably close to the predicted values confirming the validity and the adequacy of the predicted models. The experimental data were within 95% confidence interval of predicted values.

5. Comparison between MAE and conventional extraction methods

The efficiency of TPC extraction and antioxidant activityy using MAE was compared with solid/liquid extraction by maceration and stirring. The conditions of different techniques and their results are summarized in table X. The use of microwaves in the extraction of phenolic compounds from tomato seeds increased significantly the antioxidant activity($p<0.05$) and dramatically reduced extraction time to only 77s. The extraction time of MAE was far less than that of conventional methods. The MAE method isable to extract nearly 22% more TPC from tomato seeds in 1/30 of the time required for solid/liquid stirring extraction (Table X). Many studies reported in the literature have shown that applying MAE to many materials can significantly reduce extraction time compared to conventional extraction methods (Proestos and Komaitis, 2008; Spigno and De Faveri, 2009).

Many reports 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 maceration (ME).MAE techniques are excellent in terms of its extraction efficiency, technique stability and reproducibility and also the ability to retain the functional values of extracted active compounds(Chan et al., 2011).

Table X: Comparison between microwave assisted extraction and conventional extraction

	Extraction methods	**Irradiation time**	**Ethanol fraction**	
TPC	MAE	77s	29	266.97±10.70[a]
(mg GAE/100g)	Maceration	1h	29	171.39±20.24[c]
		6h	29	166.19±2.32[c]
		24h	29	145.66±4.94[c]
	Stirring	30min	29	208.11± 11.24[b]
		1h	29	171.63±9.79[c]
		2h	29	171.14±13.67[c]
DPPH (%)	MAE	76s	0	82.30±4.88[a]
	Maceration	1h	0	16.99±9.75[b]
		6h	0	14.07±2.59[b]
		24h	0	14.75±6.15[b]
	Stirring	30min	0	21.79±14.74[b]
		1h	0	18.29±8.06[b]
		2h	0	23,48±8,86[b]

Conclusion

Conclusion

An efficient microwave-assisted extraction (MAE) technique was developed to extract phenolic compounds from tomato seeds. The operating parameters were optimized using central composite design combined with response surface methodology (RSM).

RSM was successfully used to study the influence of the microwave power, irradiation time and solvent polarity on TPC and antioxidant activity of extracts. The second order polynomial model can be applied to optimise the parameters of tomato seeds extraction to obtain an extract with high phenolic compound and antioxidant capacity. Maximal values of phenolic compound and antioxydant capacity from seeds tomato were 266.97 mg GAE/100 g and 82.30 %, respectively. The optimum extraction conditions were as follows: microwave power, 600W; extraction time, 77. 22 s; liquid to solid ratio, 30mL; and ethanol fraction, 29.18 %. After method development, the extraction yield of phenolic compound and the antioxidant activity of the extract were compared with those of the extracts obtained from the conventional methods. MAE showed obvious advantages in terms of high extraction efficiency and antioxidant activity of extract within shortest extraction time. The results demonstrated that MAE could be a fast and reliable method for quantitative analysis of phenolic compounds in tomato seeds.

To conclude, use of microwave ensured the ease, and rapidity of extraction in comparison with conventional methods and the yields were better in most cases. The results obtained indicate that industrial tomato by products contains significant amounts of phenolic compounds. The phenolic compounds are known to exert antioxidant activity. Therefore, as tomato seeds are bioorganic materials and being in line with the trend for sustainability and recycling/reusing, these value adding constituents could be either isolated from the wastes to be used as natural antioxidants for the formulation of functional foods, or to serve as additives in food systems to increasing their shelf-life.

references

A

Afoakwah, A., Owusu, J., Adomako, C., Teye, E., 2012. Microwave assisted extraction (MAE) of antioxidant constituents in plant materials. Global Journal of Bioscience and Biotechnology 1, 132-140.

Ajay Shukla, Ramchandra Gupta, Prabhakar Sharma, Jain, A.P., 2013. Comparative Study of Microwave Assisted With Conventional Extraction of Calcium Sennosides from Senna Leaf. Journal of Pharmaceutical, Biological and Chemical SciencesJournal of Pharmaceutical, Biological and Chemical Sciences 4, 103.

Al-Harahsheh, M., Kingman, S.W., 2004. Microwave-assisted leaching—a review. Hydrometallurgy 73, 189-203.

Ankit,G, Madhu, N, Kothari, V., 2012. MODERN EXTRACTION METHODS FOR PREPARATION OF BIOACTIVE PLANT EXTRACTS. Journal of Applied and Natural Sciences (IJANS). 1, 8-26.

B

Balasundram, N., Sundram, K., Samman, S., 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry 99, 191-203.

Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76, 965-977.

C

Candioti, L.V., De Zan, M.M., Camara, M.S., Goicoechea, H.C., 2014. Experimental design and multiple response optimization. Using the desirability function in analytical methods development. Talanta 124, 123-138.

Chan, C.H., Yusoff, R., Ngoh, G.C., Kung, F.W., 2011. Microwave-assisted extractions of active ingredients from plants. Journal of chromatography. A 1218, 6213-6225.

D

Dahmoune, F., Boulekbache, L., Moussi, K., Aoun, O., Spigno, G., Madani, K., 2013. Valorization of Citrus limon residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction. Industrial Crops and Products 50, 77-87.

Dai, J., 2006. Microwave-assisted extraction and synthesis studies and the scale-up study with the aid of FDTD simulation. ProQuest.

Dejaegher, B., Heyden, Y.V., 2011. Experimental designs and their recent advances in set-up, data interpretation, and analytical applications. Journal of pharmaceutical and biomedical analysis 56, 141-158.

E

Eskilsson, C.S., Björklund, E., 2000. Analytical-scale microwave-assisted extraction. Journal of Chromatography A 902, 227-250.

H

Haddadi-Guemghar, H., Janel, N., Dairou, J., Remini, H., Madani, K., 2014. Optimisation of microwave-assisted extraction of prune (Prunus domestica) antioxidants by response surface methodology. International Journal of Food Science & Technology 49, 2158-2166.

Heuvelink, E., 2005. Tomatoes. CABI.

J

Jain, T., Jain, V., Pandey, R., Vyas, A., Shukla, S., 2009. Microwave assisted extraction for phytoconstituents-An overview. Asian J Res Chem 2, 19-25.

Jang, M.-J., Sheu, S.-R., Wang, C.-C., Yeh, Y.-L., Sung, K.-H., 2009. Optimization analysis of the experimental parameters on the extraction process of propolis, Proceedings of the International MultiConference of Engineers and Computer Scientists. Citeseer, pp. 18-20.

Jasionowicz, P., 2012. The chemical and genetic basis of tomato flavour. University of Nottingham.

Jiang, F., Hsieh, Y.L., 2015. Cellulose nanocrystal isolation from tomato peels and assembled nanofibers. Carbohydrate polymers 122, 60-68.

K

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.-P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry 47, 3954-3962.

Kalogeropoulos, N., Chiou, A., Pyriochou, V., Peristeraki, A., Karathanos, V.T., 2012. Bioactive phytochemicals in industrial tomatoes and their processing byproducts. LWT - Food Science and Technology 49, 213-216.

Karabegović, I.T., Stojičević, S.S., Veličković, D.T., Nikolić, N.Č., Lazić, M.L., 2013. Optimization of microwave-assisted extraction and characterization of phenolic compounds in cherry laurel (Prunus laurocerasus) leaves. Separation and Purification Technology 120, 429-436.

L

Liu, B., Peng, J., Zhang, L., Wan, R., Guo, S., Zhou, L., 2010. Optimization of preparation for Co3O4 by calcination from cobalt oxalate using response surface methodology. Chemical Engineering Research and Design 88, 971-976.

Liyanapathirana, C., Shahidi, F., 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry 93, 47-56.

M

Mallakpour, S., Rafiee, Z., 2011. New developments in polymer science and technology using combination of ionic liquids and microwave irradiation. Progress in Polymer Science 36, 1754-1765.

Mandal, V., Mandal, S.C., 2010. Design and performance evaluation of a microwave based low carbon yielding extraction technique for naturally occurring bioactive triterpenoid: Oleanolic acid. Biochemical Engineering Journal 50, 63-70.

Menéndez, J.A., Arenillas, A., Fidalgo, B., Fernández, Y., Zubizarreta, L., Calvo, E.G., Bermúdez, J.M., 2010. Microwave heating processes involving carbon materials. Fuel Processing Technology 91, 1-8.

M essak, . R., 2014 .Tomato sector in Algeria. agri benchmark Horticulture conference Italy.

Mirzaei-Aghsaghali, A., Maheri-Sis, N., 2008. Nutritive value of some agro-industrial by-products for ruminants-A review. World J. Zool 3, 40-46.

N

Naika, S., de Jeude, J.v.L., de Goffau, M., Hilmi, M., van Dam, B., 2005. Cultivation of tomato. Didigrafi Publishing. Netherlands.

Navarro-González, I., García-Valverde, V., García-Alonso, J., Periago, M.J., 2011. Chemical profile, functional and antioxidant properties of tomato peel fiber. Food Research International 44, 1528-1535.

P

Prakash Maran, J., Manikandan, S., 2012. Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (Opuntia ficus-indica) fruit. Dyes and Pigments 95, 465-472.

Proestos, C., Komaitis, M., 2008. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. LWT - Food Science and Technology 41, 652-659.

R

Radojkovića, M., ZEKOVIĆA, Z., JOKIĆB, S., VIDOVIĆA, S., 2012. Determination of optimal extraction parameters of mulberry leaves using Response Surface Methodology (RSM). Romanian Biotechnological Letters 17, 7295-7308.

Raffo, A., Malfa, G.L., Fogliano, V., Maiani, G., Quaglia, G., 2006. Seasonal variations in antioxidant components of cherry tomatoes (Lycopersicon esculentum cv. Naomi F1). Journal of Food Composition and Analysis 19, 11-19.

Razdan, Maharaj K, and Autar K Mattoo.2006.Genetic improvement of solanaceous crops: Tomato. Volume 2: CRC Press.

S

Sainju, U.M., Dris, R., Singh, B., 2003. Mineral nutrition of tomato. Food, Agriculture & Environment 1, 176-184.

Spigno, G., De Faveri, D.M., 2009. Microwave-assisted extraction of tea phenols: A phenomenological study. Journal of Food Engineering 93, 210-217.

Strati, I.F., Oreopoulou, V., 2011. Process optimisation for recovery of carotenoids from tomato waste. Food Chem 129, 747-752.

T

Toor, R.K., Savage, G.P., 2005. Antioxidant activity in different fractions of tomatoes. Food Research International 38, 487-494.

V

Vági, E., Simándi, B., Vásárhelyiné, K.P., Daood, H., Kéry, Á., Doleschall, F., Nagy, B., 2007. Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. The Journal of Supercritical Fluids 40, 218-226.

Vallverdú-Queralt, A., Medina-Remón, A., Casals-Ribes, I., Andres-Lacueva, C., Waterhouse, A.L., Lamuela-Raventos, R.M., 2012. Effect of tomato industrial processing on phenolic profile and hydrophilic antioxidant capacity. LWT - Food Science and Technology 47, 154-160.

W

Wang, L., Weller, C.L., 2006. Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology 17, 300-312.

Witek-Krowiak, A., Chojnacka, K., Podstawczyk, D., Dawiec, A., Pokomeda, K., 2014. Application of response surface methodology and artificial neural network methods in modelling and optimization of biosorption process. Bioresource technology 160, 150-160.

Y

Yang, B., Liu, X., Gao, Y., 2009. Extraction optimization of bioactive compounds (crocin, geniposide and total phenolic compounds) from Gardenia (Gardenia jasminoides Ellis) fruits with response surface methodology. Innovative Food Science & Emerging Technologies 10, 610-615.

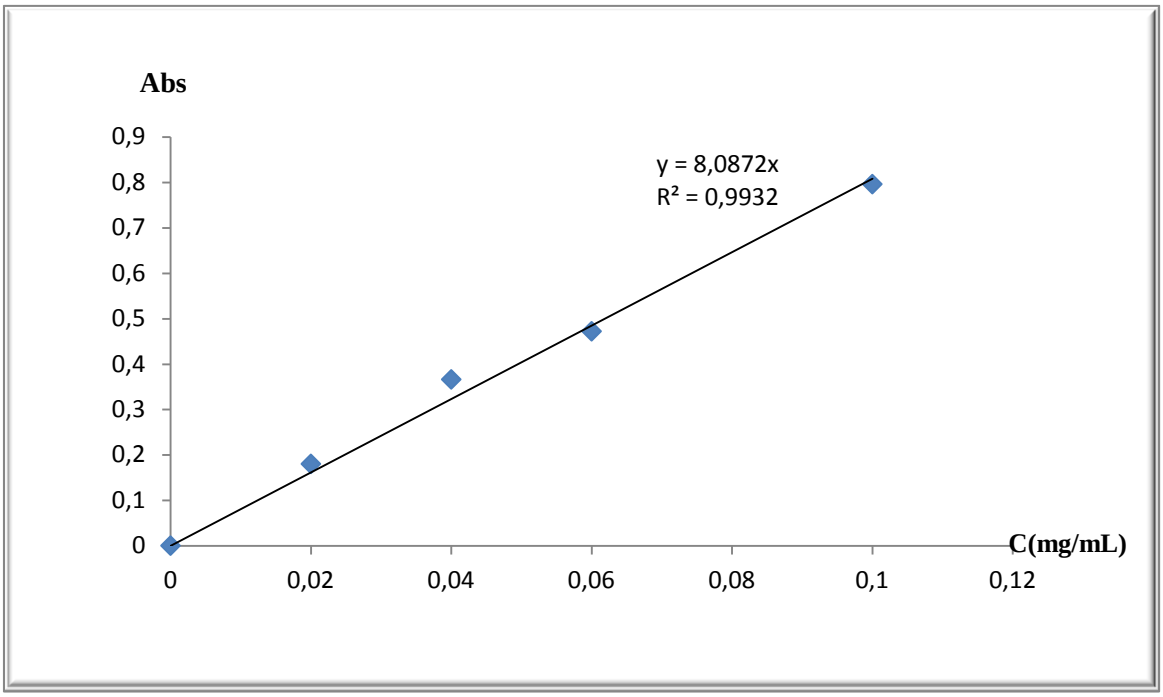
Z

Zaghdani, A.S., 2002. Effect of pre-sowing seed treatments for quality of cucumber, pepper, tomato and pea seed. Szent István Egyetem.

Zhang, H.-F., Yang, X.-H., Wang, Y., 2011. Microwave assisted extraction of secondary metabolites from plants: Current status and future directions. Trends in Food Science & Technology 22, 672-688.

Zhong, K., Wang, Q., 2010. Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. Carbohydrate Polymers 80, 19-25.

Appendix I: Calibration curve of TPC



Abstract

In this study, microwave assisted extraction was used to extract the phenolic compounds from tomato seeds. The effect of three independent variables, microwave power, irradiation time and solvent on the total phenolic compounds yield and the antioxidant activity was estimated by using response surface methodology (RSM). The optimum conditions extractions to obtain a better yield of TPC are 600 W for microwave power, 77.22 s for irradiation time and 29.18 %(v/v) for ethanol fraction. MAE is more efficient than conventional extraction method to obtain TPC from tomato seeds. The experimental values were reasonably close to the predicted values confirming the validity of the predicted models.

Keywords: Optimization**,** Microwave Assisted Extraction, Total phenolic compounds, tomato seeds, response surface methodology.

Résumé

Dans cette étude, l'extraction assistée par micro-ondes (EAM) a été utilisée pour extraire les composés phénoliques à partir des grains de tomate. L'effet des trois variables indépendantes, puissances de micro-ondes, le temps d'irradiation et le solvant sur la teneur des composés phénolique totaux et l'activité antioxydante a été évalué en utilisant la méthodologie de réponse de surface (MRS). Les conditions optimales de EAM permettant d'obtenir un meilleur rendement en CPT sont 600 W pour la puissance du micro-onde, 77.22s pour le temps d'irradiation et éthanol 29.18 %. EAM est plus efficace que les méthodes conventionnelles pour l'obtention des CPT à partir des graines de tomates. Les valeurs expérimentaux sont proche des valeurs prédites ce qui confirme la validité du model mathématique.

Mots clés : Optimisation, Extraction Assistée par Micro-ondes, Composés phénoliques totaux, Les grains de tomate, Méthodologie de Réponse de Surface.