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 des Sciences Alimentaires
Filière : Sciences Biologique
Spécialité : Sciences Alimentaires
Option : Bioprocédés et technologie alimentaire

Réf :.....

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

MASTER

Thème

Optimisation de l'extraction par sonication des antioxydants des épluchures de la figue de barbarie

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Soutenu le : **13 Juin 2016**

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Année universitaire : 2015 / 2016

Remerciements

Tout d'abord nous remercions le Bon Dieu de nous avoir donné la force et le courage d'achever ce modeste travail.

Notre profonde gratitude va à notre promotrice Mme GUEMGHAR pour l''honneur qu'elle nous a fait en nous encadrant, pour ses précieux conseils, orientations et la confiance placée en nous et pour sa gentillesse et sa bonne humeur.

Nous remercions le Pr. MADANI.K de nous avoir accueillis au sein de son laboratoire 3BS tout au long de la durée de notre stage.

Nous remercions également Mme BELKHIRI et MINA GUEMGHAR pour leurs présence leurs gentillesse et leurs aide si précieuse

Nous remercions les Membres du jury, Melle ISAADI.O pour avoir accepté d'examiner notre travail et Mme OUCHEMOUKHE.N de présider le jury.

Nos remerciements les plus sincères et les plus chaleureux vont, également, à toute la promo Sciences alimentaires 2016 et à toutes les personnes qui ont cru en nous et qui nous ont aidés de près ou de loin à la réalisation de ce modeste travail.

Sabrina et Latifa.

Dédicaces

Je dédie ce travail aux deux personnes qui illuminent chaque jour de ma vie, mes chères parents que dieu les protèges et les gardes en parfaite santé.

A mes deux adorables sœurs adorée Nassima et Lydia pour leurs présence et leurs soutient.

A mes deux frèresAmirouche et Mokrane.

A toute ma famille oncles, tantes ; cousins et cousines et ma grand-mère Yemma tata.

A mon adorable binôme et amie et a toute sa famille

A tous mes amis(e) de près ou de loin Djidji, Ouazna, Hamid, Nassima, Hanane, Samy, Nafaa, Sora, Ghania, Lynda et àtoute la promo sciences alimentaires 2016 spécialement Messi, Djemai, Abdou, Nassima, Souhila, Kenza, Zahra, Hamza, Brahim, Farida, Lynda, Lynda, Baya, Fairouz et Katia.

Merci

Latifa.

Dédicace

Tous les mots ne sauraient exprimer la gratitude, l'amour, le respect, la reconnaissance, c'est tout simplement que : Je dédie ce travail à :

A Ma tendre Mère : Tu représentes pour moi la source de tendresse et l'exemple de dévouement qui n'a pas cessé de m'encourager. Tu as fait plus qu'une mère puisse faire pour que ses enfants suivent le bon chemin dans leur vie et leurs études.

A Mon très cher Père : Aucune dédicace ne saurait exprimer l'amour, l'estime, le dévouement et le respect que j'ai toujours pour vous. Rien au monde ne vaut les efforts fournis jour et nuit pour mon éducation et mon bien être. Ce travail et le fruit de tes sacrifices que tu as consentis pour mon éducation et ma formation le long de ces années.

A mon très cher mari OMAR : Tes sacrifices, ton soutien moral et matériel m'ont permis de réussir mes études. Que ce travail soit témoignage de ma reconnaissance et de mon amour sincère et fidèle.

A mes deux chers frères : Yacine, Zahir et sa femme souraya.

A ma sœur : sylia.

A mes chers beaux-parents.

A mes chère belle sœurs, mes chers beaux-frères.

A ma copine Tifa.

A toute mes copines avec les quelle que j'ai passé mes plus belle années de mon cursus Soura, Ghania, Ouazna, Latifa, Zahra, Salima, Thafath(dhawéya), Siham, Sylia et Simou

A toute la promo Sciences alimentaires 2016

Sabrina

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List of abbreviations

Pre: Predict

AA: Antioxidant activity

Adj: Adjustement

BBD: Box-Behnken design

CCD: Central Composite design

CV: Coefficient of variation

GAE : Gallic acid equivalent

LSD: Least Significant Difference

MW: Molecular weight

OFI: *Opuntia ficus indica*

RSM: Response surface methodology

R^2: Coefficient of determination

rpm : Rotation per minute

TPC: Total phenolic compound

UAE: Ultrasound assisted extraction

2D : 2 Dimensional

3D: 3 Dimensional

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Introduction

Opuntia ficus indica (OFI) is native to Mexico but it has been domesticated in other countries and proliferates today on dry and stony lands of the five continents. The first economic importance of this plant relies on the production of edible fruits **(Chougui *et al,*. 2015).**

This kind of cactus is widely cultivated in the world due to its adaptability to difficult growing conditions; arid and semi arid zones **(Cejudo-Bastante *et al,*. 2014).**

Mexico is the world's largest producer of prickly pear with annual productions of around 428,300 Tones/year and also possesses a vast genetic variability **(Stintzing *et al,*. 2005).** In Mediterranean countries, Morocco is the largest producer with an area of more than 46,000 ha for growing prickly pear, 5% of Moroccan arboreal heritage; production is estimated at 57,000 tons reaching an average yield of 1.2 tonnes / ha. In Tunisia, the yield is 250 to 300 quintals of fruit per hectare **(Walali-Loudyi 1998).** In Algeria, OFI develops on the Mediterranean shore and particularly in Kabylie region but its cultivation is marginalized, its consumption is still seasonal and its production is always traditional (**Nadia *et al,*. 2015)**.

A commercialization of the cactus-pear based on its antioxidant properties could generate competitive advantages that may turn into business opportunities and the development of new products (**Sumaya-Martínez *et al,*. 2010)**.

Recent studies in the varieties of European and Asian cactus pears have shown notable antioxidant activities that significantly reduce oxidative stress in patients and may help in preventing chronic pathologies. Betalain pigments contained in these cactus pears have shown beneficial effects on the redox-regulated pathways involved in cell growth and inflammation **(Wolfram *et al,*. 2003, Tesoriere *et al,*. 2004, Tesoriere *et al,*. 2005, Siriwardhana *et al,*. 2006).**

Several studies have suggested that the total antioxidant activity of the cactus pear is due to its content of vitamin C, polyphenolics, flavonoid compounds (e.g., kampeferol, quercetin, and isorhamnetin), pigments (betalains) and taurine**, (Butera *et al,*. 2002, Castellar *et al,*. 2003, Galati *et al,*. 2003, Tesoriere *et al,*. 2003, Chavez-Santoscoy *et al,*. 2009)**.

Betalains are water-soluble pigments. Two betalains derivatives are present in cactus-pear: betacyanins, which give the red-purple color, and betaxanthins, which give a yellow-orange color. These pigments show important antioxidant activities without toxic effects in humans **(Sumaya-Martínez *et al,*. 2011).**

These functional compounds are found in both the pulp and peel of prickly pear, but it has been shown that the peel of many fruits contains higher concentrations of phytochemical compounds than the pulp **(Escobedo-Avellaneda *et al*,. 2014)**. Due to these properties, prickly pear can be considered as a functional fruit that offers numerous health benefits when is consumed as fresh or processed product **(Jimenez-Aguilar *et al*,. 2015).**

Extraction represents the primary step in getting extract from plants **(Haddadi-Guemghar *et al*,. 2014)**. Recently, some new extraction techniques have been developed which include: ultrasound-assisted extraction, microwave-assisted extraction, accelerated solvent extraction and ultra high pressure extraction. Thus, ultrasonic-assisted extraction is a more desirable technique for the extraction of plant ingredient, **(Chen *et al*,. 2015)**. The use of ultrasound has attracted considerable interest as an alternative approach to the traditional extraction methods **(Li *et al*,. 2007).**

The extraction conditions may not be generalized due to the diverse nature of natural antioxidants existing in different plant materials **(Wettasinghe and Shahidi 1999).**. For this, the optimization of experimental conditions is very important. Response surface methodology (RSM) is a useful tool for optimizing the chemical process; it is an efficacy mathematical and statistical technique for analysis of empirical models that describes the effect of independent variables and their interactions on response variables **(Hammi *et al*,. 2015).**

The aim of this study was to determine the optimal parameters in ultrasound assited extraction (UAE) process such as temperature, amplitude, extraction time and liquid to solid ratio on the TPC, betalains and antioxidant activity contents in skins of prickly pear using RSM.

Theorical part

Chapter I
General information of prickly pear

I.1. Origins and geographic distribution of *Opuntia ficus indica*

Prickly pear *(Opuntia ficus indica)* is native of South America but is also found in Africa, Australia, south of Europe and Asia. The Cactaceae family is found in Mediterranean basin, Middle East, South Africa and India. The plant is now found in most parts of the world **(Nharingo and Moyo 2016).**

Opuntia cactus is a native species of Mexico. It was one of the food bases for the indigenous populations. It was introduced in southern Spain after Columbus's first expedition to the New World. There after, it was spread throughout the Mediterranean Basin by the Spanish conquerors in the 16th and 17th century. The culture of *Opuntia* covers about 200,000 ha in North Africa 30–40% in Tunisia. *Opuntia* cactus was introduced in Morocco in the early 17th century from Spain (**Stintzing *et al,*. 2001)**.

I.2. Description of *Opuntia ficus indica*

The cactus pear fruit, also known as prickly pear, tuna, or fico d'india, is an oval, elongated berry of 67-216g weight. They offer a wide spectrum of colors from white, yellow, orange, red and purple based on betalains. The thick pericarp is covered with small-barbed spines hosting a juicy pulp with 150-300 non edible seeds. The latter account for 3-7% on a weight basis, followed by the pericarp and mesocarp (36-48%) and the edible pulp (39-64%). Thus, cactus pear may be divided into three fractions that may be exploited for commercial processing: seeds, peel and pulp **(Moßhammer *et al,*. 2006).**

I.3.Chemical composition of the fruit of *Opuntia spp*

The fruit constitutes an important source of nutritive, useful elements for the human health such as rough proteins, fat contents, fibers, vitamins, minerals and lipids…. whose contents vary according to the compartment and also depend on the cultivar, of the cultivation methods, of the period of lighting, the climate and the season of harvest. Table I recapitulates the principal components of the 3 compartments of *Opuntia spp* **(Chougui., 2014).**

Table I: Principal chemical components of the fruits of *Opuntia spp* **(Ramadan and Mörsel 2003a, Ramadan and Mörsel 2003b, Habibi 2004, Piga 2004, Moßhammer *et al*,. 2006, Tounsi *et al*,. 2011).**

Parameters	Peel	Pulpy juice	Seeds
Fresh weight (%)	33 – 55	43 – 67	2 – 10
Water (%)	85 – 94,40	84,7 – 90,33	5 – 6
Color	Green, orange, red, Crimson	White, red, yellow, orange, crimson	Brown
Minerals	Potassium, calcium	Potassium, calcium, Magnesium	Potassium, calcium
Vitamins	Vit. C, Vit. E	Vit. C, Vit. E	Vit. E
Amino-acids	Nongiven	Proline, taurine	Glutamic acid, aspartic acid
Sugars	Glucose	Glucose, fructose	Glucose, xylose
Fibers	Cellulose, pectin	Pectin, rhamno-galacturonane, 50% of nonpectic substances	Cellulose, arabinanes, rhamnogalacturonanes
Organic acids	Nongiven	Citric acid	Nongiven
Lipids	γ - linolenic acid α - linolenic acid	Linoleic acid, Palmitic acid	Linoleic acid, palmitic and oleic
Sterols	β – sitosterol, campesterol	β – sitosterol, campesterol	β – sitosterol, campesterol
Phenolic	Quercetin, cinnamic acid, rutin, epigallocatechine	Quercetin, kaempferol, isorhamnetine	Nongiven
Pigments	Betacyanins, betaxanthines	Betacyanins, betaxanthines	Nongiven

I.4. Use of *Opuntia ficus indica*

Opuntia ficus indica is used in several fields:

- Industrial use:

Prickly pears are often used to make candies, jelly, or drinks such as vodka or lemonade **(Maran *et al,*. 2013)**, or used in food flavourings and colourings**(Kuti 2004).** It is also used in cosmetic. The prickly pear juice enter in the composition of certain soaps and protective creams against the sun. The seeds can be crushed to extract a cream for the skin **(Chougui 2014).**

- medicinal and therapeutic use:

These fruits have shown antiulcerogenic, antioxidant, anticancer, neuroprotective, hepatoprotective, and antiproliferative activity. Moreover, prickly pear may be used for the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostate hypertrophy **(De Leo *et al,*. 2010).**

- Other use:

The plant constitutes a good food for the cattle like fresh fodder or is stored in the form of ensilage by providing digestible energy, water and vitamins. It is used for this end in the form of fruits not accepts by the man, peels or rackets removed from their cut spines of small pieces mixed with other food of cattle **(Chougui 2014).**

I.5. Skins of prickly pears

Skins represent a large proportion of the whole fruit (from 40% to 50%) and constitute a source of bioactive compounds, notably phenolics, flavonoids and betalains **(Chougui *et al,*. 2015).**

Chapter II
Antioxidants of prickly pear

II.1. Definition of antioxidants

Antioxidants are a series of bioactive compounds commonly used to preserve the quality of food products by protecting them against oxidation rancidity. They also protect the human body from many chronic cardiovascular diseases, cancers, and ageing by capturing free radicals **(Samaram *et al,*. 2014)**

II.2. Antioxidants

II.2.1. Phenolic compounds

Phenolic compounds like Quercetin, cinnamic acid, rutin, epigallocatechine Isorhamnetine kaempferol, are secondary metabolites, ubiquitous widely exist in nature and food-industry by-products. They are differentiated from one another by their structure and molecular weight, and the resulting physicochemical and biological properties. Due to this enormous variety, there are reports of more than 10000 phenolic molecules and the list continues expanding, **(Dahmoune *et al,*. 2015)**

The concentration of polyphenols varies depending on the nature of the sample analyzed (whole fruit or juice, red or yellow). The content of red polyphenols in prickly pear is about 15.34 ± 0.73 mg/kg of juice and 17.81 ± 0.10 mg/kg for whole fruit. In the yellow prickly pear the polyphenols content was 15.03 ± 1.36 mg/kg for juice and 15.03 ± 1.36 mg/kg for whole fruit **(Khatabi *et al,*. 2011).**

II.2.2.Betalains

Betalainsare a class of pigments, yellow Chromoes-alkaloids (betaxanthins). Some cultivars of the prickly pear are characterized by a deep red color caused blush by the presence of betacyanin pigments **(Khatabi *et al,*. 2011).**

The highest levels of betalains from the pulp and peel of *O. ficus-indica* together amounted to 113.9 mg/100 g fresh fruit, which is comparable to the most intensely coloured red beet hybrids. Considering that systematic selection of prickly pear for higher pigment yield has not been performed, breeding of cultivars with intensely coloured pulp appears to be a promising horticultural task **(Stintzing *et al,*. 2001).**

II.2.3. Vitamin C

Ascorbic acid (vitamin C) is a major metabolite in higher plants. This occurs in all compartments including the cell wall. Ascorbic acid is involved in cell division, cell expansion, cell growth, defense against oxidative stress, photosynthesis, and antioxidant

metabolism. Vitamin C is necessary in the everyday diet to prevent scurvy **(Mandl *et al*,. 2009) .**

The total ascorbic acid content ranged from 10 to 111 mg/g fresh weight in purple-skinnedcactus pear fruits and from 23 to 792 mg/g fresh weight in red-skinned fruits, which are considerably higher than the average vitamin C contents in some common fruits, such as peaches, grapes and apple **(Kuti 2004)**.

II.2.4. Flavonoids

The flavonoids set up a group of more than 6.000 natural compounds which are almost universal at the vascular plants. They constitute pigments responsible for colorings yellow, orange and red of various vegetable bodies. **(Ghedira 2005)**

Total flavonoid contents ranged from as low as9.8 ± 3.0 mg/g fresh weight in yellow-skinned cactus pear fruits to as high as 93.5 ± 12.4 mg/g fresh weight in purple-skinned cactus pear fruits. It appears that Opuntia cactus pear fruits contain common flavonoids to other fruits and vegetables**(Kuti 2004).**

Chapter III
Ultrasound-assisted extraction of antioxidants

III.1. Definition of ultrasound

Ultrasound is defined as sound waves having frequency that exceeds the hearing limit of the human ear (~20 kHz). Some animals utilize ultrasound for navigation (dolphins) or hunting (bats) using the information carried by back-scattering sound waves. Ultrasound is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure the safety of food products. Ultrasound is applied to impart positive effects in food processing such as improvement in mass transfer, food preservation, assistance of thermal treatments and manipulation of texture and food analysis**(Awad *et al,*. 2012)**

Ultrasound is commonly employed for enhancing physical processes such as cleaning, emulsification, degassing, crystallization, extraction, etc. and for accelerating/performing chemical reactions. In recent years, ultrasound has become a highly useful method for performing a wide range of chemical reactions and processes, including chemical synthesis, materials production and water treatment**(Guesmi *et al,*. 2013)**

III.2. Advantages of the ultrasound

The application of ultrasound as a laboratory based technique for assisting extraction from plant material is widely published. Several reviews have been published in the past to extract plant origin metabolites**(Vilkhu *et al,*. 2008).**

Ultrasound-assisted extraction (UAE) has been widely used for the extraction of nutritional material, such as lipids, proteins, flavoring, essential oilsand bioactive compounds (e.g., flavonoids, carotenoids, and polysaccharides. Compared with traditional solvent extraction methods, ultrasound extraction can improve extraction efficiency and extraction rate, reduce extraction temperature, and increase the selection ranges of the solvents. In comparison with other extraction methods such as supercritical fluid extraction and microwave-assisted extraction, ultrasound equipment is simpler and economically cheaper **(Sun *et al,*. 2011).** Ultrasound was applied in extraction of plant materials because of enhancement of yield and shortening of extraction time**(Lieu 2010).** Ultrasound assisted extraction is adjustable to be used with polar and non-polar solvents in various temperatures**(Samaram *et al,*. 2015)**

III.3. Principle of the ultrasound

Various novel extraction techniques have been developed for the extraction of antioxidant secondary metabolites including ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, and accelerated solvent extraction. Among these techniques, ultrasound-assisted extraction is a simple, efficient and inexpensive alternative. It is more effective at extracting secondary metabolites due to the acoustic cavitation effect produced in the solvent by the passage of ultrasonic waves which can lead to the destruction of cells and enhance the contact surface area between solid and liquid phases. These effects permit better penetration of the solvent into the sample increasing the extraction yield of secondary metabolites**(Hammi *et al*,. 2015).**

Chapter IV
Response surface methodology

IV.1. Definition

Response surface methodology (RSM) is an effective statistical technique for optimizing complex extraction processes. It is a collection of statistical method that has been successfully used to determine the effects of multiple parameters, alone or in combination, on response variables and also predict their behavior under given sets of conditions. The main advantage of RSM is to reduced number of experimental trials needed to evaluate multiple parameters and their interactions. It is widely used in optimizing the extraction process variables, such as polysaccharides, phenolic, anthocyanins and proteins from different materials **(Li *et al,*. 2012)**

IV.2.Terminology

The definition of some key words concerning the response surface methodology:

- **Experimental design:**

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

- **Coded factor levels:**

Experimental design 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 be varied for the systems under study and is chosen on the basis of the literature information or earlier knowledge **(Candioti *et al,*. 2014).**

- **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).**

- **Responses or dependent variables:**

Response 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 or residual carbon, and final acidity, among others **(Dejaegher and Vander Heyden 2011)**

- **Residual:**

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).**

- **Response surface design:**

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. **(Dejaegher and Vander Heyden 2011).**

IV.3. Steps for RSM application

Some stages in the application of RSM as an optimization technique are as follows:

- Screening of variables:

Numerous variables may affect the response of the system studied, and it is practically impossible to identify and control the small contributions from each one. Therefore, it is necessary to select those variables with major effects. Screenings designs should be carried out to determine which of the several experimental variables and their interactions present more significant effects **(Dejaegher and Vander Heyden 2011)**.

- Choice of the experimental design:

After determining the significant factors, the optimum operation conditions are attained by using more complex experimental designs such as central composite designs (CDD), Box-Behnken design (BBD) or Doehlert matrix (DM) **(Ferreira *et al*,. 2007).**

- Determination of the model equation:

The simplest model which can be used in RSM is based on a linear function. For its application, it is necessary that the responses obtained are well fitted to the following equation: $$Y= \beta_0 \sum^{k} \beta_i X_i + \varepsilon \qquad (1)$$

Where k is the number of variables, $\beta_0$is the constant term, $\beta_i$represents the Coefficients of the linear parameters, $X_i$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. This way, a model for a second-order interaction presents the following terms: $$Y= \sum \beta_i X_i + \sum_{1 \leq i \leq j} \beta_{ij} X_i X_j + \varepsilon \qquad (2)$$

Where β_{ij} 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:

$$Y=\beta_0 + \sum \beta_i X_i \sum^{k}_{i=1} \beta_{ii} X_i^2 + \sum^{k}_{1\leq i\leq j} \beta_{ij} X_i X_j + \varepsilon \qquad (3)$$

Where β_{ii} 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 **(Bezerra *et al,*. 2008).**

- Evaluation of the fitted model:

The mathematical model found after fitting the functions 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)

Lack of fit test in 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 experimental data if it presents a significant regression and a non-significant lack of fit **(Bezerra *et al,*. 2008).**

- Determination of the optimal:

The research must find the best operational condition inside the studied experimental condition by visual inspection**(Bezerra *et al,*. 2008)**

Pratical part

Materials and methods

I.1.Taxonomy of *Opuntia ficus indica*

The classification reported by Wallace and Gibson in 2002 is the following one:

Kingdom: Plantae

Under kingdom: Spermaphyte

Division: Angiosperme

Classify: Dicotylédone

Subclass: Caryophyllale

Family: Cactaceae

Subfamily: Opuntiadieae

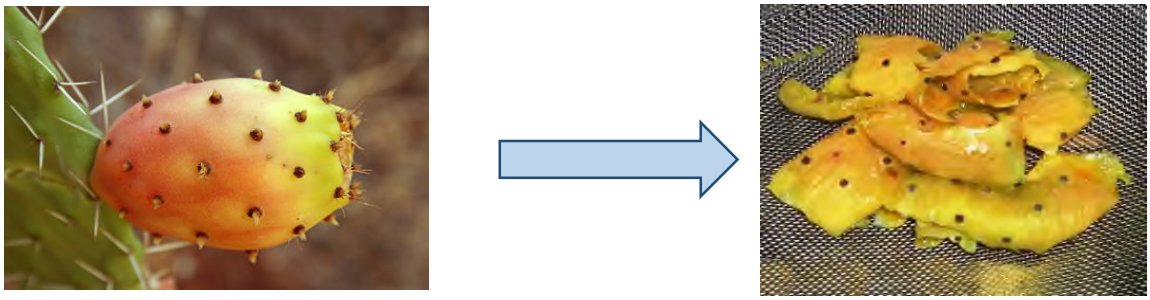
Genus: *Opuntia*

Species: *Opuntia ficus indica*

I.2. Plant materials

Fruits were harvested in Barbacha (located at the south of Bejaia, in Algeria, Latitude: 36 ° 34 '0' 'north Longitude: 4 ° 58 '0' 'East.), during the month of September 2015.

The fruit were washed and thorns removed. Prickly pear was immediately peeled in order to separate the skins from the fruits (The skins were manually removed).



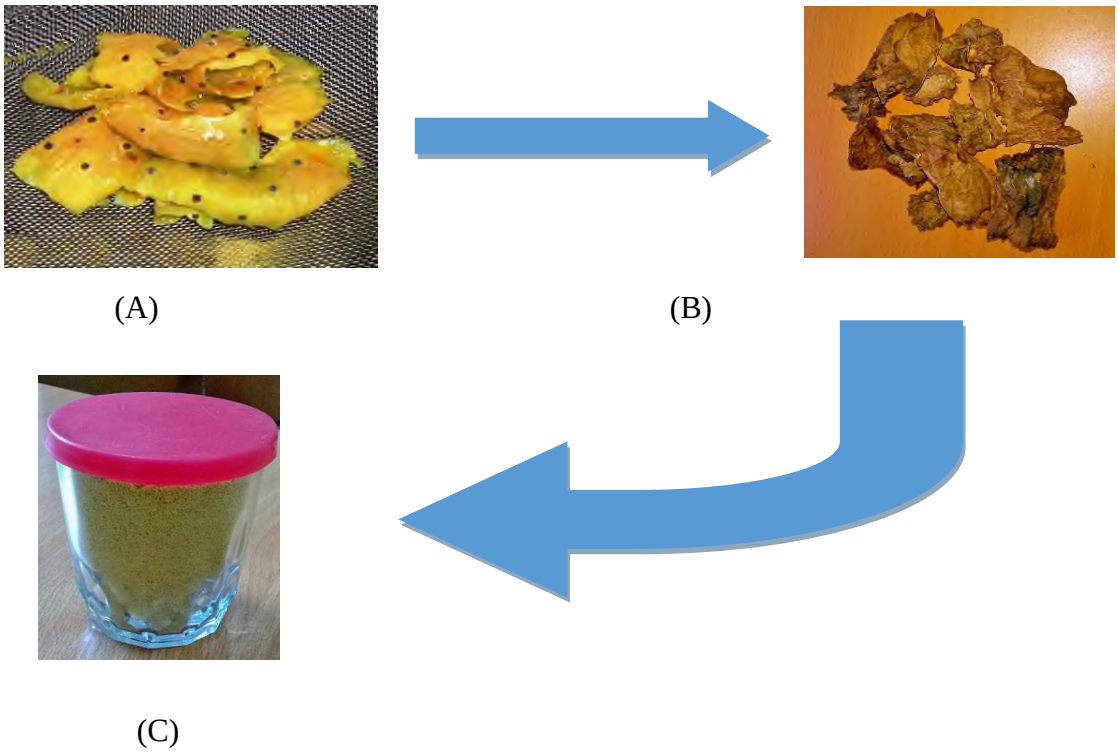
(A) (B)

Figure 1: Photography (A): fruit of prickly pear, (B): skin of prickly pear.

I.3. Preparation of samples

The skin of the prickly pear were recovered and dried in stove at a temperature of 50° for 48 hours.

The dried skin were ground with an electrical grinder (IKA model A11Basic.Germany) and the powder obtained was passed through a 500 µm sieve.



(A) (B)

(C)

Figure 2: Photography of: skin of prickly pear (A), dried skin of prickly pear (B), dried powder of prickly pear skin (C).

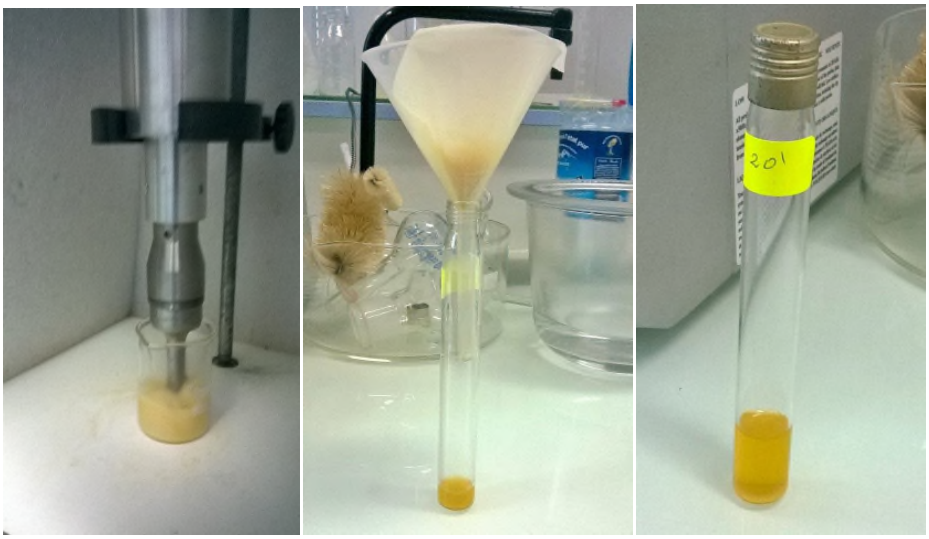
I.4. Extraction procedure

Extraction is the first key step to isolate natural bioactive compounds from plants and materials.

I.4.1. Ultrasound assisted extraction

Extractions were carried out in an ultrasound (BIOBLOCK SCIENTIFIC Vibra cell 75115, USA) at 20 kHz, POWER 500 WATTS. The samples (0,5g) were placed into beaker (50 mL) (figure A5). Samples were extracted by following the experimental disgn (table III). Ethanol was selected as extraction solvent due to its low toxicity. The mixtures were filtered through Whatman filter paper (figure B6) and the filtrates were centrifuged at 5000 rpm for 15 min and the supernatants were collected and used to determine the TPC, betalains and antioxidant activity.

Figure 3: Photography of ultrasound



(A) (B) (C)

Figure 4: (A): ultrasound extraction; (B): filtration; (C): the extract.

I.4.2. Conventional extraction

Extraction by stirring and maceration were done in order to compare with ultrasound assisted extraction:

- ❖ Stirring

The extraction method used for dried samples had as follows: 20 mL of 20% Ethanol solvent was added to 0.5 g of dried sample in a beaker (150 mL). The mixtures were stirred carefully during 15 minutes, 30 minutes and 2 hours. Each extraction was repeated two times.

After each extraction, the mixture was filtered through Whatman filter paper, the filtrates were centrifuged at 5000 rpm for 15 minutes then recovered and stored at 4°C until analyzes.

.

Figure 5: Photography of extraction by stirring.

❖ Maceration

The extraction method used for dried samples had as follows: 20 mL of 20% Ethanol solvent was added to 0,5 g of dried sample in a beaker during 1hour, 2 hours, 6 hours and 24 hours. Each extraction was repeated two times.

After each extraction, the mixture was filtered through Whatman filter paper, the filtrates were centrifuged at 5000 rpm for 15 minutes then recovered and stored at 4°C until analyzes.

I.5. Determination of Betalains

Betacyanins and betaxanthins content was determined according to Castellar *et al.* and Stintzing *et al*, were reported like mg equivalent betanin/L and mg equivalent indicaxanthin/L, respectively. Betacyanins were detected at 538 nm and betaxanthins at 480 nm, according to the next equation(**Sumaya-Martínez *et al*,. 2011)**:

Betacyanins or betaxanthins content [mg/L] =

$$betalains(mg/L) = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L}$$

Where:

A = absorbance at 535 or 480 nm;

DF = dilution factor;

MW = molecular weight;

Ɛ= extinction coefficient;

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

For betacyanin the extinction coefficient is 60,000 L/ (mol.cm) and MW = 550 g/mol.

For betaxanthins the extinction coefficient is 48,000 L/ (mol.cm) and MW = 308 g/mol.

Total betalains = Total betacyanins +Total betaxanthins

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

I.6. Determination of total phenolic compounds (TPC)

The concentration of total phenolic compounds was determined spectrophotometrically, using the Folin–Ciocalteu total phenol procedure. Gallic acid standard solutions were prepared at 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml. The extracts (1mL) and the gallic acid standards (1 mL) were transferred to test tubes. 1mL of 0.2 N Folin–Ciocalteu reagent were added to each test tube and mixed using a vortex mixer. After 5min, 1ml of 9.0% (w/v) Na2CO3 (Sodium carbonate) in water were added and mixed. Absorbance at 750 nm was determined using a spectrophotomètre ((UV-Vis Spectrophotometer, Spectro Scan 50) after 30 min at room temperature and in dark. The concentration of TPC in the extracts was determined by comparing the absorbance of the extract samples to that of the gallic acid standard solutions. Total phenolic content (TPC) was expressed as mg gallic acid equivalents (GAE) per 100g dry weight **(Ballard *et al,*. 2010).**

I.7. Anti-radical activity (DPPH)

Two milliliter of 0.2 mM DPPH in ethanol were mixed with 200 µL of samples. The mixture was shaken with vortex and kept in the dark for 30 min at room temperature and absorbance was measured at 510 nm. The experiment was performed in triplicate. The DPPH radical scavenging rate was expressed as the following equation **(Chen *et al,*. 2015)**:

$$Scavenging\ rate\ \% \quad = \quad \left(1 \quad - \quad \frac{As - Ac}{A}\right) \times \quad 100$$

Where:

As is the absorbance of the sample reaction solution;

Ac is the absorbance of the solution including 2 mL of ethyl alcohol and 2 mL of sample,

A is the absorbance of the solution including2 mL of DPPH and 2 mL of ethyl alcohol.

I.8. Preliminary study

I.8.1.Effect of the time on the extraction process

In the first approach, the effect of the time on the yield of extraction was separately investigated in single factor experiments in order to evaluate the time of the process. All other parameters were kept constant: amplitude at 60%, solvent at 30% ethanol (v/v) and liquid to solid ratio at 40ml/g. The temperature was taken at the end of each extraction.

I.8.2. Kinetic model

A first order kinetic model of the extraction of betalains and total phenolic compounds by ultrasonication was applied, in preliminary study, to evaluate the time of extraction.

$$\frac{dY}{dt} = k[Y]$$

$$\frac{dY}{Y} = kdt$$

$$\int_{t}^{teq} \frac{dY}{Y} = k \int_{t}^{teq} dt$$

$$Ln\ \frac{Yt}{Yeq} = -kt + b$$

$$\frac{Yt}{Yeq} = e^{-kt+b}$$

Where Yt and Yeq (mg/g) are the yields of Indicaxantin, betanin, total betalain and TPC at any time t (min) and at the equilibrium, respectively; k (mg/g/min) is the mass transfer coefficient of the whole extraction process.

I.9. Statistical analysis

Response surface methodology (RSM) was used to determine the optimal conditions for extraction. RSM was performed using the Design Expert software (version DX10) program.

I.9.1. Application of central composite design (CCD)

The Central composite design (CCD) is the most popular second-order design which was introduced by Box and Wilson **(Natarajan *et al*,. 2011)**. It is a factorial or fractional factorial design with centre points and star points. The star points are added to estimate the curvature. The factorial design points in CCD contribute to the estimation of the interaction terms. The axial points contribute in a large way to the estimation of quadratic terms. Without the axial points, only the sum of the quadratic terms can be estimated. The factorial points do not contribute to the estimation of quadratic terms. The centre runs provide an internal estimate of error (pure error) and contribute toward the estimation of quadratic terms. The areas of flexibility in the use of central composite design reside in the selection of axial distance (α) and the number of centre runs (nc). The choices of these parameters are very important. The choice of α depends to a greater extent on the region of operability and region of interest. The choice of nc often has an influence on the distribution of variance in the region of interest. The axial distance value α is chosen to maintain rotatability and it depends on the number of experimental runs in the factorial portion of the central composite design **(Natarajan *et al*,. 2011)**.

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, β_{ii} the quadratic coefficient, β_{ij} the interaction coefficient of variables i and j, and X_i and X_j 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 response variable at its optimal 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 II.

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

Code	Amplitude %	Time (min)	Ethanol fraction (%)	Liquid to solid ratio (mL)
-1	20	10	0	20
0	60	20	30	40
1	100	30	60	60

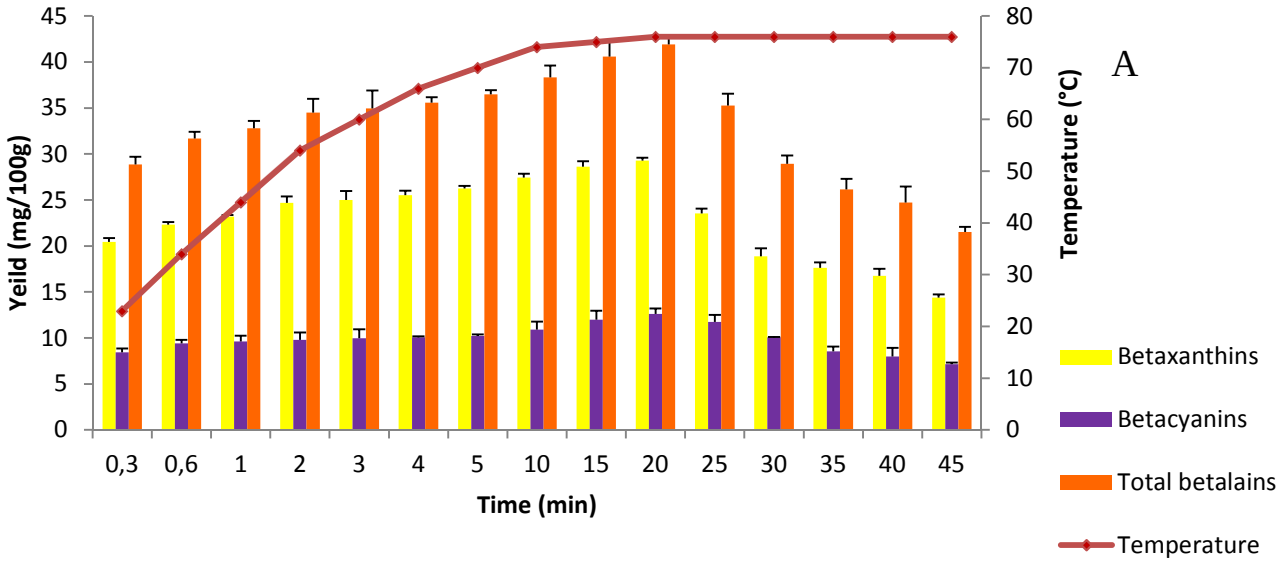
Results and discussion

II.1. Preliminary study

II.1.1. Effect of the time on the extraction process

Figure 6 shows the evolution of the extraction yields and temperature with the time of the ultrasonication. The experimental data shows that the yields of extraction of betalains (fig. 6A) increased with the increasing of time until 20 min and then decreased, however the TPC yield increased until15min and then decreased (fig. 6B). The evolution of the extraction of both betalains and TPC is done in two phases; phase of extraction and then the phase of degradation. The yields of extraction seem related to the temperature of the system. At the first phase of extraction (from 0.3min to 20min) good linear correlation was observed between the temperature of the system and the extraction yield of betaxhantins (r=0.96), betacyanins (r=0.84), total betalains (r=0.94) and TPC (r=0.96). The extraction using ultrasounds is attributed to the propagation of ultrasound pressure waves through the solvent and resulting in cavitation phenomena and thermal effects which can result in disruption of cell walls, particle size reduction, and enhanced mass transfer across cell membranes **(Shirsath *et al*,. 2012).** A large amount of energy can be produced from the conversion of kinetic energy of motion which increased the temperature of the system **(Azmir *et al*,. 2013).**

The increasing in the temperature improve the diffusion of the analyte from the cell by enhancing the mass transfer however high temperatures with prolonged extraction times can decrease the yield of extraction by degradation and destruction of analytes.



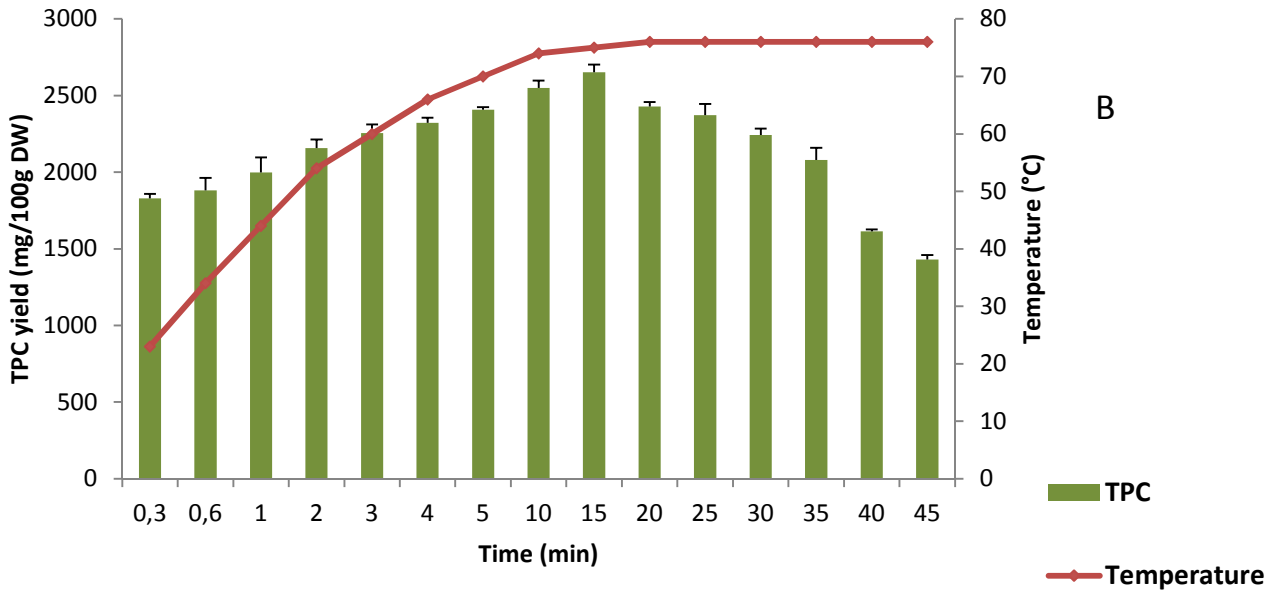


Figure 6: Effect of the ultrasonication time on the yields of betalains (A) and TPC (B) and on the evolution of the curve of temperatures.

II.1.2. Extraction kinetics

The experimental data for betaxanthins, betacyanins, total betalains and TPC consistent with first-order kinetic models (y= aln (t) + b), with correlation coefficients of 0.992, 0.948, 0.975 and 0.986, respectively **(**Fig. 7). The yield of extraction of betaxanthins increased dramatically as the extraction time was increased from 0 min to 20 min, however large increases in the yields of betacyanins were observed in the first 10 min, followed by smaller increases with further increases in the extraction time. For TPC the maximum of the extraction yield was obtained after 15min. Therefore, 10–30 min was selected in the following experiments.

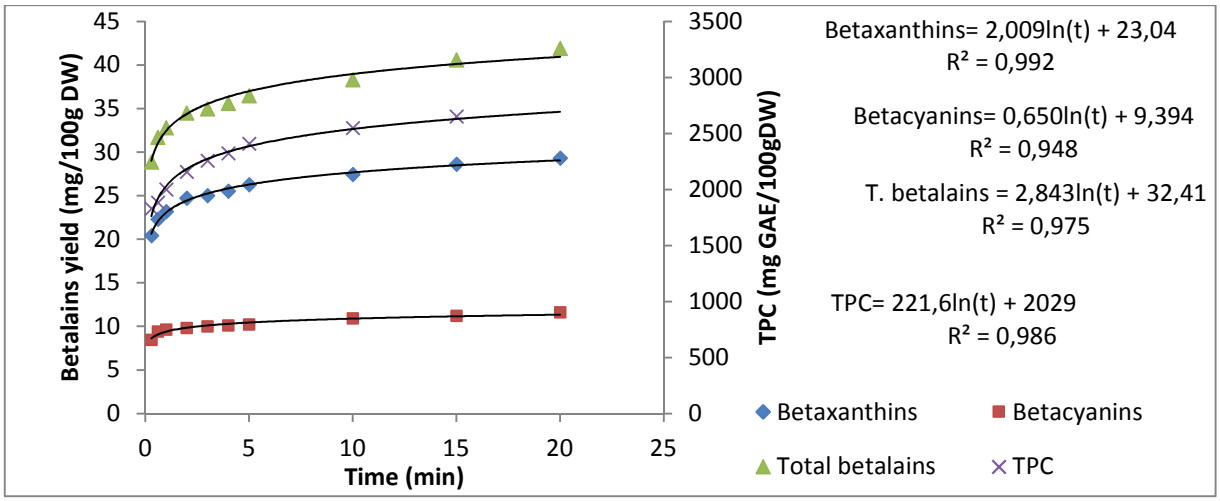


Figure 7. Dynamic curves for betalains and TPC.The points represent actual values, and lines represent fitting behavior predicted by first order models.

II.2. Optimization by RSM

II.2.1. Building models and statistical analysis

In this study four parameters amplitude (A), extraction time (B), ethanol concentration (C) and liquid to solid ratio (D) that affect ultrasound assisted extraction were optimized by RSM using CCD. The extraction efficiency was evaluated by measuring the extraction yields of TPC, total betalains and antioxidant activity. Following the experimental design by CCD, 30 runs of the extraction including six replicates of the centre point were conducted (Table III).

Table III: Four-factor, three levels central composite design used for response surface methodology (RSM) and experimental data of the investigated responses of O. ficusindica skin.

Amplitude	Extraction time	Ethanol concentration	Solid to liquid ratio	TPC (mg GAE/100g DW)	TB (mg GAE/100g DW)	AA (%)
100	10	60	20	1152.91	35.3765	84.3255
100	10	0	60	1495.6	39.6784	77.5828
20	30	0	20	1533.54	26.1642	76.509
60	10	30	40	1412.18	45.1088	69.0056
20	10	60	20	1351.5	34.5511	79.1919
20	10	60	60	1310.47	34.5352	64.548
100	30	0	60	1384.6	22.5395	70.4033

Suite						
20	20	30	40	1422.51	28.487	70.4631
60	20	30	40	1443.87	39.5669	77.1989
60	20	30	40	1456.68	38.5743	78.5758
60	20	30	40	1412.18	44.3068	78.185
20	30	60	20	1572.15	24.2665	77.0793
100	20	30	40	1345.98	27.4412	71.7803
20	30	60	60	1528.02	33.6751	71.8081
20	10	0	60	1343.24	30.0307	75.6885
60	20	30	40	1484.6	40.3203	80.0862
100	30	60	20	1389.28	23.2068	77.9138
60	30	30	40	1467.34	30.2662	70.4033
60	20	0	40	1628.73	49.6422	85.4103
100	10	0	20	1522.51	39.2434	69.2907
20	30	0	60	1379.08	27.5158	79.7413
60	20	30	60	1439.06	42.6817	89.3324
60	20	30	40	1434.25	41.7865	76.2726
20	10	0	20	1550.09	36.1892	69.3046
60	20	30	40	1484.6	45.7463	80.6954
100	30	0	20	1384.6	26.9406	72.7024
60	20	30	20	1445.28	38.5629	96.2448
60	20	60	40	1653.49	61.3327	81.8081
100	30	60	60	1405.28	28.9521	69.7274
100	10	60	60	1252.21	47.5762	75.146

Fitting the models for total betalains, TPC, and antioxidant activity of the *O. ficus indica* skin extracts is crucial to elucidate how precisely the RSM mathematical model can predict the responses. The analyze of variance of each responses and the statistical parameters shown in table IV and V indicate that the model is adequate to predict the extraction of antioxidants by ultrasonication from *O. ficus indica.* The probability *p-values* of all regression models were <0.05 which indicates a high significance of the model. The

R^2 values for TPC, TB and antioxidant activity were 0.93, 0.93 and 0.92 respectively indicating a statistically significant agreement between the observed and predicted responses and that the model equations can reliably predict the experimental results. The Adj R^2 and the Pred R^2 of TPC, TB 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 all dependant variables. 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, TB and antioxidant activity was within the acceptable range.

The significance of each coefficient was determined using *p-value* in table IV. 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 A, B, C, D, AC, AD, BC, CD, A^2, B^2, C^2 are significant model terms for TPC, B, AB, CD, A^2, B^2, C^2 are significant model terms for TB and D, AB, AC, CD, A^2, B^2, D^2 are significant model terms for antioxidant activity.

After neglecting all the non significant terms (p>0.05), the fitted quadratic models for TPC, TB and AA are given in Eqs (1), (2) and (3), respectively.

$$\textit{TPC} = +1472.27-36.5\mathbf{*\ A} + 36.28\ \mathbf{*\ B} -33.70 * \mathbf{C} - 20.23 * \mathbf{D} -33.98 * \mathbf{AC}+33.42* \mathbf{AD} +66.07* \mathbf{BC} +26.14 * \mathbf{CD} -107.60* \mathbf{A^2}-52.09 * \mathbf{B^2}+149.25 * \mathbf{C^2} \quad (1)$$

$$\textit{TB} = +42.37 - 5.48\ \mathbf{*\ B} -2.28 * \mathbf{AB} + 2.25 * \mathbf{CD} -15.07 * \mathbf{A^2} - 5.35 * \mathbf{B^2}+12.44 * \mathbf{C^2} \quad (2)$$

$$\textit{AA} = +79.52 -1.58 * \mathbf{D} - 2.00 * \mathbf{AB} +1.61 * \mathbf{AC} - 3.30 * \mathbf{CD} - 9.42* \mathbf{A^2}- 10.84 * \mathbf{B^2} +12.24* \mathbf{D^2} \quad (3)$$

Table IV: ANOVA for the effect of amplitude, extraction time, ethanol fraction and liquid to solid ratio on TPC, total betalains and antioxidant activity using a quadratic response surface model.

Source	***TPC***		***Total betalains***		***Antioxidant activity***	
	Coef estimate	*p-value*	*Coef estimate*	*p-value*	*Coef estimate*	*p-value*
Model	*1472.27*	*< 0.0001*	*42.38*	*< 0.0001*	*79.52*	*< 0.0001*
A-Amplitude	*-36.54*	*0.0010*	*0.86*	*0.2778*	*0.25*	*0.2778*
B-Time	*36.29*	*0.0011*	*-5.49*	*< 0.0001*	*0.12*	*< 0.0001*
C-Ethanol concentration	*-33.70*	*0.0020*	*1.42*	*0.0841*	*0.27*	*0.0841*
D-Liquid to solid ratio	*-20.24*	*0.0402*	*1.26*	*0.1210*	*-1.59*	*0.1210*
AB	*-19.81*	*0.0558*	*-2.28*	*0.0132*	*-2.00*	*0.0132*
AC	*-33.99*	*0.0029*	*-0.026*	*0.9748*	*1.61*	*0.9748*
AD	*33.43*	*0.0032*	*0.59*	*0.4814*	*-0.067*	*0.4814*
BC	*66.08*	*< 0.0001*	*2.692E-003*	*0.9974*	*-0.89*	*0.9974*
BD	*-0.44*	*0.9636*	*0.35*	*0.6706*	*-0.21*	*0.6706*
CD	*26.15*	*0.0153*	*2.26*	*0.0141*	*-3.31*	*0.0141*
A^2	*-107.61*	*0.0004*	*-15.07*	*< 0.0001*	*-9.42*	*< 0.0001*
B^2	*-52.09*	*0.0445*	*-5.35*	*0.0183*	*-10.84*	*0.0183*
C^2	*149.25*	*< 0.0001*	*12.45*	*< 0.0001*	*3.06*	*< 0.0001*
D^2	*-49.69*	*0.0539*	*-2.42*	*0.2503*	*12.24*	*0.2503*

Table V: Statistical parameters of the model fitting.

Statistical parameters	**TPC**	**TB**	**AA**
Std. Dev.	38.23	3.25	2.61
Mean	1436.19	36.14	76.55
C.V. %	2.66	9.00	3.41
Lack of fit	1.022E+005	786.71	608.00
R-Squared	0.9310	0.9324	0.9215
Adj R-Squared	0.8666	0.8693	0.8483
Pred R-Squared	0.6783	0.6647	0.5335
AdeqPrecision	17.374	14.884	14.208

II.2.2. Response surface analysis

The 3D response surface is the graphical representation of regression equation. It provides a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. Figure 8 shows the effects of the independent variables and their mutual interaction on the extraction yield of TPC. Fig 8-e is a response surface plot showing the effect of amplitude and extraction time on the TPC yield at the fixed ethanol fraction of 30% and liquid to solid ratio of 40ml/g. The amplitude was shown as a linear and negative quadratic effect on the yield (p<0.001). The yield of extraction increased up to about 60% followed by a decline with further increase of amplitude. Similar effect was shown for extraction time; the yield of TPC extraction increased up to about 17 min and then decreased with further increase of extraction time. Ultrasound offers a mechanical effect allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between the solid and liquid phases. As a result, heat and mass transfer are enhanced and the solute diffuses more rapidly from the solid phase into the solvent **(Meullemiestre *et al*,. 2016).**

The increase of amplitude and the extraction time leads to increasing in the temperature of the extraction solvent. 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. These results are in agreement with results obtained in preliminary study which shown that the yield of TPC extraction decrease when the temperature of the extraction achieving 76°C at amplitude of 60% and after extaction time of 20 min.

Ethanol concentration and its interaction with extraction time are the most parameters affecting the extraction yield of TPC by ultrasoniction. Positive interaction was found between ethanol concentration and extraction time which mean that at 30 min of extraction time, ethanol 30% give the best yields however at less concentration of ethanol the best yields is obtained with shorter extraction times.

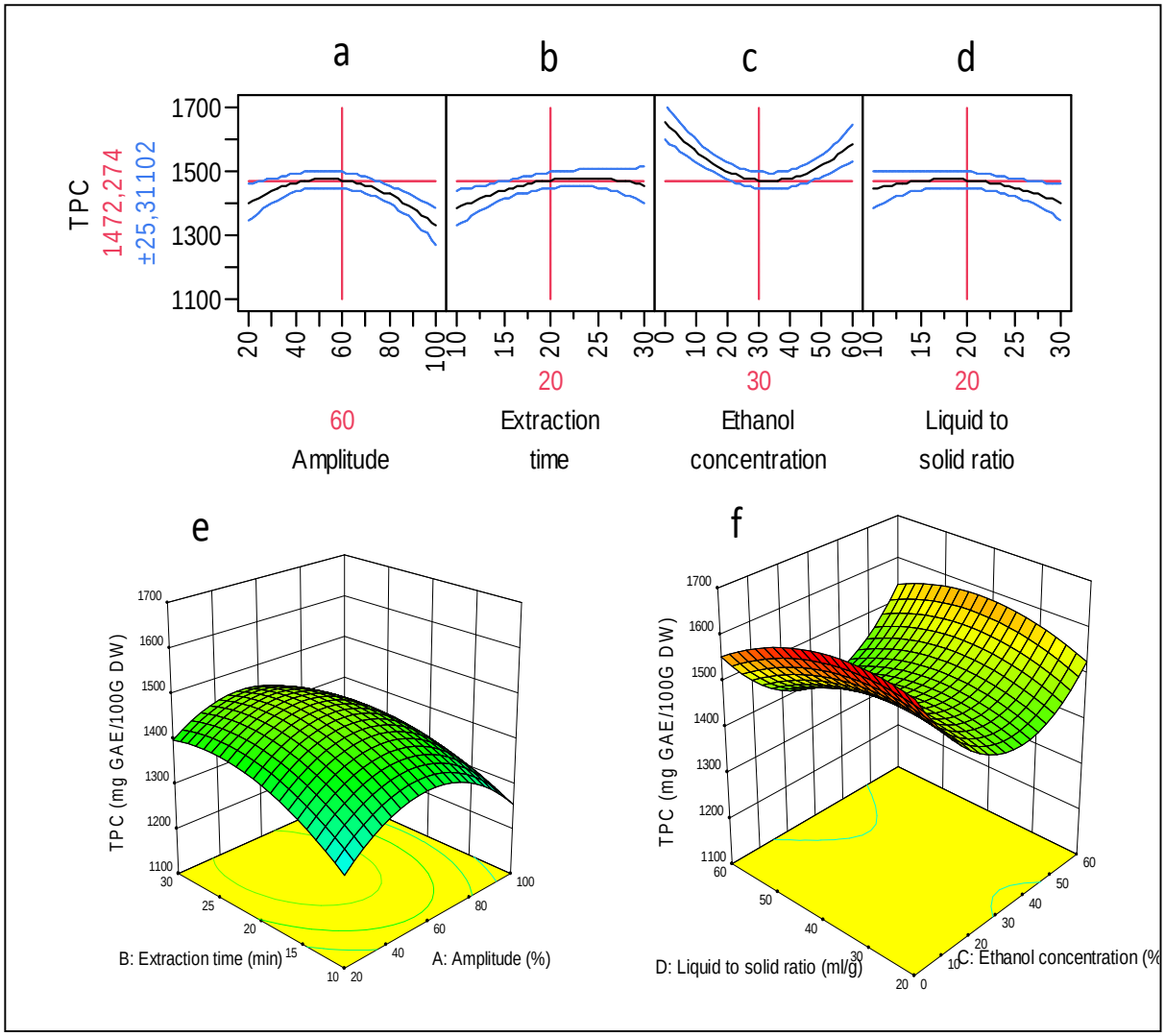


Figure 8: Impact of amplitude (20–100 %), extraction time (10–30min), ethanol concentration (0-60%) and liquid to solid ratio (20-60 mL/g) on TPC. The 2D impact of amplitude, extraction time, ethanol concentration and liquid to solid ratio were expressed in a–d; while their 3D effects were shown in e–f

Figure 9 shows the effects of the independent variables and their mutual interaction on the extraction yield of total betalains. The extraction yield of betalains increased with the increasing of amplitude up to 63% and then decreased for further amplitude. The most parameters affecting the betalains extraction is amplitude (quadratic effect) followed by extraction time (linear effect). Negative interaction was observed between amplitude and extraction time which mean that the maximum desirability is obtained when an increase of the one of this factor is followed by the decrease of the second factor.

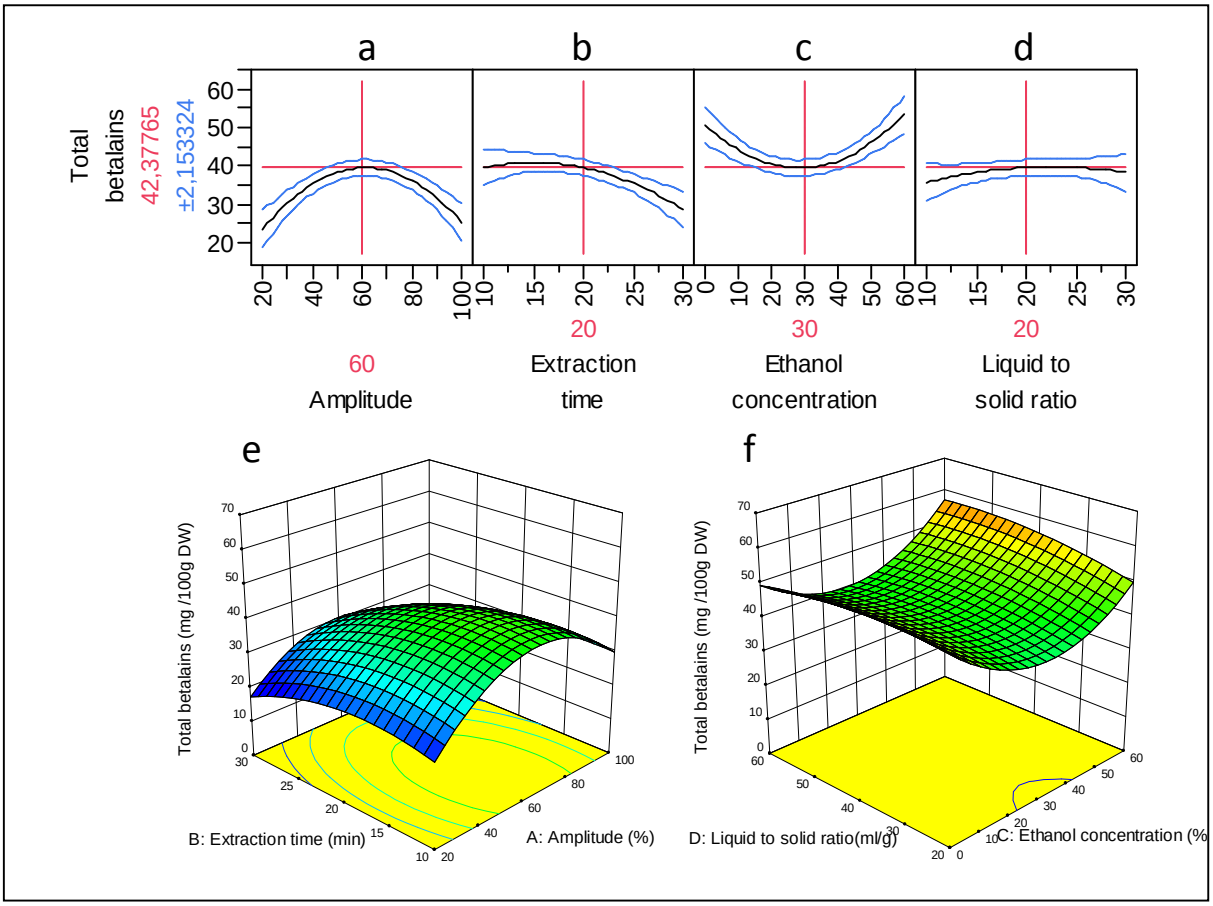


Figure 9: Impact of amplitude (20–100 %), extraction time (10–30min), ethanol concentration (0-60%) and liquid to solid ratio (20-60 mL/g) on total betalains. The 2D impact of amplitude, extraction time, ethanol concentration and liquid to solid ratio were expressed in a–d; while their 3D effects were shown in e–f

Figure 10 shows the effects of the independent variables and their mutual interaction on the antioxidant activity. The quadratics effects of liquid to solid ratio, amplitude and extraction time were the most parameters affecting the antioxidant activity of the *O. ficusindica* extracts. The increase of the irradiation time from 10 min to 20 min, over a amplitude of 60% improved antioxidant activity up to 10%.

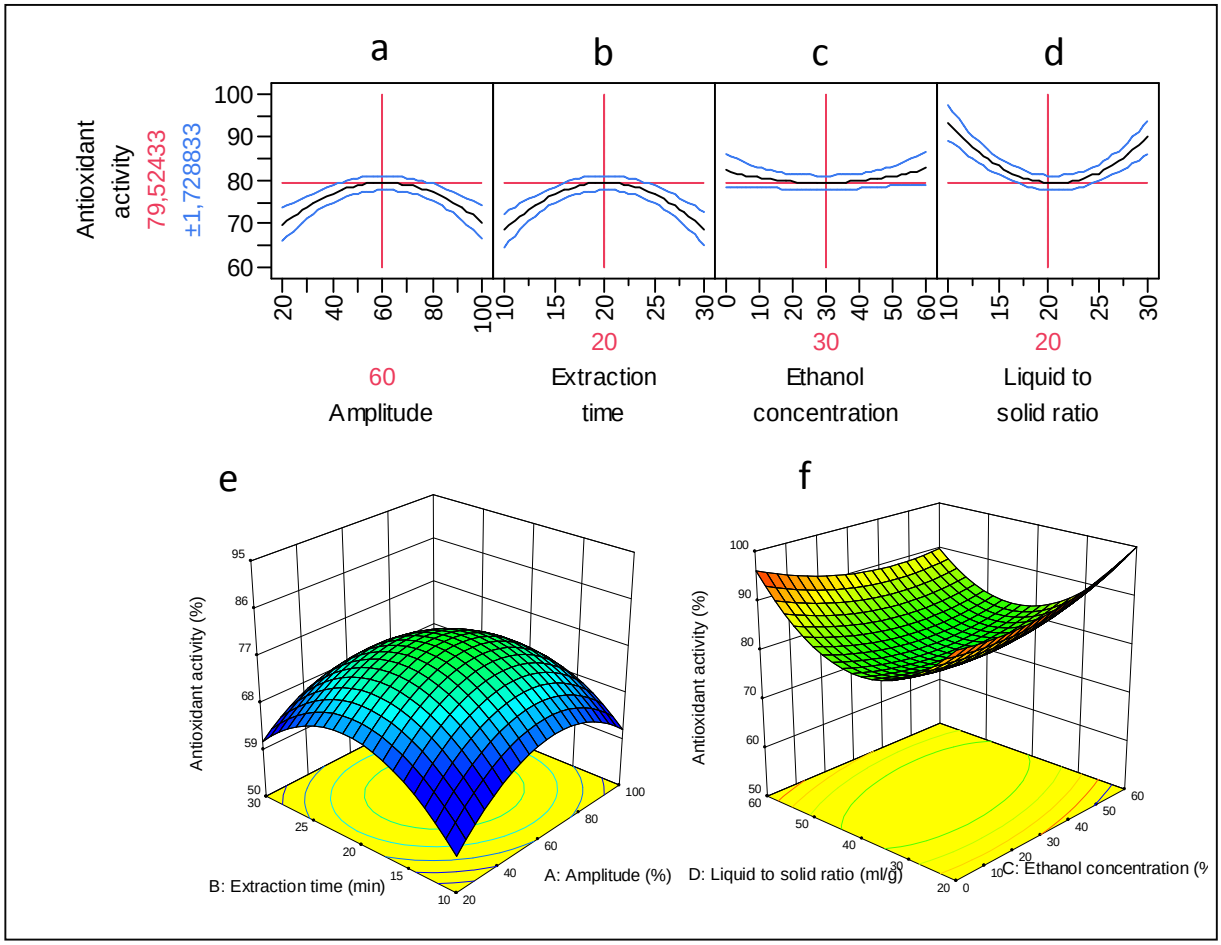


Figure 10: Impact of amplitude (20–100 %), extraction time (10–30min), ethanol concentration (0-60%) and liquid to solid ratio (20-60 mL/g) on antioxidant activity. The 2D impact of amplitude, extraction time, ethanol concentration and liquid to solid ratio were expressed in a–d; while their 3D effects were shown in e–f

II.3. Optimal extraction conditions

Using a quadratic model to describe the experimental we optimized three experimental variables for maximal extraction of total phenolic compounds, total betalains and antioxidant activity from *O. ficus indica*. The results of the optimization are summarized in table VI. When comparing the optimal conditions based on TPC to those obtained for total betalains , it was found that the optima of amplitude and extraction time were closely related. However, there was a difference in the ethanol concentration required for optimal extraction of TPC and total betalains.

According to the results of this study the content of phenolic compounds is of 1625. 46 ± 76.54 mg GAE/100g DW. This result is close to that found by **Chougui *et al*,. (2015)**,

which is of 1512.58 ± 31.5 mg GAE/100 g DW, and that found by **Jimenez-Aguilar *et al,*. (2015)**which is of (1534,0 ± 36,0 mg GAE/100g DM).

From the available literature it appears that is particularly rich in total phenolic in comparison to other fruit andvegetable peels. Indeed, OFI peel phenolic content is 2 times higher than fruit peel of the related species Opuntia stricta and several times higher than commonly consumed fruits and vegetables like lemon (435.76 mg/100 g DM), pineapple (120.87 mg/100 g DM), potato (240 mg/100 g DM) and tomato (462 mg/100 g DM), This makes OFI peel a very interesting source for bioactive compound re-valorization **(Al-Weshahy *et al,*. 2013).**

Table VI: Optimum conditions for ultrasonication assisted extraction of TPC (mg GAE/100g), total betalains (mg GAE/100g) and antioxidant activity (%) from *O. ficus indica skin*

	Amplitude (%)	Extraction time (min)	Ethanol concentration (%)	Liquid to solid ratio (ml/g)	Prediction	Experimental
TPC(mgGAE/100g DW)	57	17	0	15	1670.64 ± 54.95	1625.46 ± 76.54
TB (mg /100g DW)	63	15	60	27	58.89 ± 4.69	54.95 ± 4.79
AA (%)	64.24	20	60	10	99.71 ± 4.58	96.74 ± 7.56

II.4. Validation of the models

The optimized conditions obtained by RSM were used to validate the predictive model of extraction for TPC, total betalains and antioxidant activity from *O. ficus indica* skin. According to the results represented in table VI, the content of TPC, total betalains and antioxidant activity after optimization are 1625.46 ± 76.54 mg GAE/100g DW, 54.95 ± 6.75 mg/100g DW and 96.74 ±7.56 (%) respectively are close to that predicted by the software which is of 1670,64 ± 54,95 mgGAE/100g DW, 58.89 ± 4.69 mg/100g DW and 99,71 ± 4,58 (%) of powder of the peels of prickly pear. what leads us to confirm the validation of the model.

Table VI 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.

II.5. Comparison between ultrasound assisted extraction and conventional extraction methods

The efficiency of TPC, total betalains extraction and antioxidant activity (DPPH) using UAE was compared to solid/liquid extraction by maceration and stirring. The conditions of different techniques and their results are summarized in **table VII**. The use of ultrasounds in the extraction of bioactif compounds from *O. ficus indica* significantly increased the yield of extraction ($p<0.05$) and dramatically reduced extraction time to only 17min for TPC, 15min for total betalains and 20 min for antioxidant activity.When the results abtained were compared we observed that the prolonged in the extraction time in the conventional extraction methods leads to the decreasing in the yields of the extractions in both TPC and total betalains. This maybe due to the oxydation of the bioactives compounds during the extraction. However, sonication leads to an increase in the mass transfer of the bioactifs compounds from matrix to solvent and this effect is maximum at short sonication times. Similar results were reported by **Rodriguez (2008)** who found that extraction times between 15 and 20 min give the maximum yield of TPC. The UAE method was able to extract nearly 32% and 15% more TPC and total betalains respectively than conventionnal method from *O. ficus indica* skin (Table VII).

Table VII: Comparison between ultrasonication assisted extraction and conventional extraction methods. Results are reported as means ± S.D. Same letters in the same column refers to means not statistically different according to ANOVA and LSD test.

Extraction methods	Extraction time	TPC (mg GAE/100g DW)	Total betalains (mg GAE/100g DW)	Antioxidant activity (%)
UAE	Optimal extraction conditions	1625.46 ± 76.54a	54.32 ± 6.75a	96.74 ± 7.56a
Liquid to solid extraction by stirring	15 min	1075.68 ± 93.62b	45.59 ± 5.60b	63.48 ± 6.79b
	30 min	1097.74 ± 0.55b	46.19 ± 1.52b	76.18 ± 2.95b
	1h	994.32 ± 25.35d	40.77 ± 0.05bc	44.84 ± 4.23d
	2h	915.71 ± 54.61d	38.36 ± 1.13cd	42.75 ± 6.98d
Liquid to solid extraction by maceration	1h	1041.21 ± 1.95b	35.66 ± 0,84cd	50.68 ± 7.77c
	2h	1068.79 ± 37.06b	35.68 ± 3,13cd	51.24 ± 10.52c
	6h	1031.55 ± 7.80c	32.33 ± 2,76cd	42.29 ± 7.47d
	24h	987.42 ± 19.50d	31,38 ± 3,32d	48.83 ± 1.18 cd

Conclusion

Conclusion

In the present work, we report for the first time, the use of UAE by statistical method based on the response surface methodology (RSM) in order to identify and quantify the variables which may maximize the yield of TPC, betalains and antioxidant activity of prickly pear skins.

RSM employing central composite design was successfully used to determine the optimal conditions such as amplitude, extraction time, liquid to solid ratio and ethanol concentration for extraction under ultrasound.

The optimal conditions obtained by RSM for the extraction of TPC, betalains and antioxidant activity from skins of prickly pear under ultrasonication include the following parameters: for TPC, amplitude 57%, extraction time 17 min, ethanol concentration 0% and liquid to solid ratio 15 mL/mg with a maximal values 1702mg GAE/100g DW. For betalains, amplitude 63%, extraction time 15min, ethanol concentration 60% and liquid to solid ratio 27 mL/mg with a maximal values 61 mg/100g DW. For antioxidant activity, amplitude 64,24%, extraction time 19,64 min, ethanol concentration 60% and liquid to solid ration 10mL/mg with a maximal value of 96,74%. These data suggest that the interaction of ethanol concentration and the extraction time are the most effective parameters to extract the phenolic compounds, the most parameters affecting betalains from peels of prickly pear were amplitude and the extraction time and the most parameters affecting the antioxidant activity were liquid to solid ratio, amplitude and extraction time.

Extraction by ultrasound is much more frequent than conventional extraction methods because of the increase the selection ranges of the solvents, ultrasound equipment is simpler, enhancement of yield and shortening of extraction time.

Our results also suggested that the antioxidants from prickly pear should be explored as a novel natural antioxidants for use in functional foods or medicine.

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Appendix

Appendix I: Some varieties of prickly pear fruit.

Opuntia ficus indica

Opuntia dillenii

Appendix II: Main species and varieties of prickly pear.

Table I: Main species and varieties of prickly pear.

Species	Variety
O. atropes Rose	Blanco
O. ficus-indica (L.) Miller	Milpa Alta
O. ficus-indica	Atlixco
O. ficus-indica	Copena V1
O. ficus-indica	Copena F1
O. ficus-indica	Moradilla
O. ficus-indica	Blanco
O. ficus-indica	Negro
O. ficus-indica	Blanco w/ spines
O. ficus-indica	Polotitlan
O. ficus-indica	Alba
O. ficus-indica	Lutea
O. ficus-indica	Asperma
O. ficus-indica	Piriforme
O. ficus-indica	Serotina
O. ficus-indica	Italiana
O. ficus-indica	Villanueva
O. ficus-indica	Jalpa
O. inermis De Candolle	Tlaconopal
O. robusta Wendland	Tapon
O. streptacantha Lemaire	Cardon
N. cochenillifera (L.) Salm-Dyek	Tamazunchale

Appendix III: Chemical structure of some antioxidants.

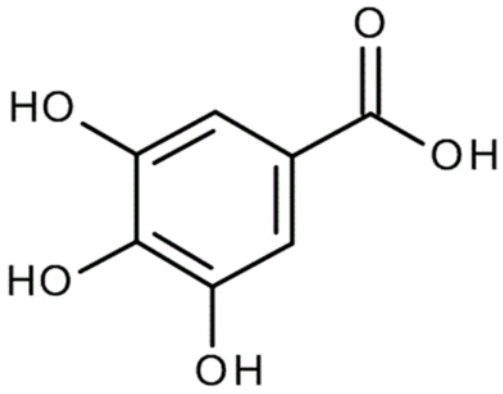

Figure 1: Chemical structure of Gallic acid

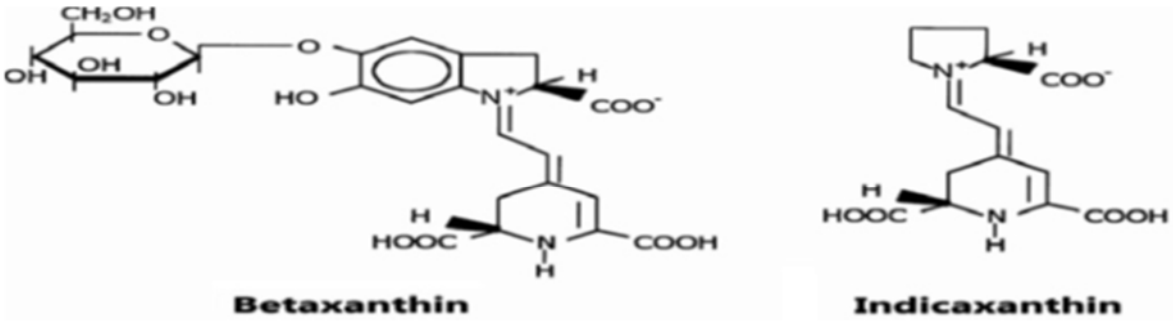

Figure 2: Chemical structure of Betaxanthin and Indicaxanthin **(Khatabi *et al*,. 2011).**

Appendix IV: Composition of prickly pear fruit**.**

Table I: Mineral composition of prickly pear fruit (mg/100 g, dry matter)

	Pulp	Skin	Seed
Ca	163	2090	258
Mg	76.1	322	208
Na	7.77	< 0.85	< 0.83
K	559	3430	275
P	0.063	0.064	110
Fe	16.5	8.31	12.1
Cu	< 0.78	< 0.85	< 0.83
Zn	1.55	1.70	4.16
Mn	6.99	72.9	< 0.83
Mb	< 0.31	< 0.34	< 0.33

Table II: Carbohydrate composition of prickly pear fruit (% dry matter)

	Pulp	Skin	Seed
Saccharose	0.22	2.36	0
Glucose	35.0	21.0	0
Fructose	29.6	2.89	0
Raffinose	0	0	0
Galactose	0	0	0
Mannose	0	0	0
Stachyose	0	0	0
Xylose	0	0	0

Table III: Fiber composition of prickly pear fruit (% of total fiber)

	Pulp	Skin	Seed
Hemicellulose	15.5 ± 0.45 b	20.8 ± 0.55 a	9.95 ± 0.58 c
Cellulose	14.2 ± 1.07 c	71.4 ± 1.99 b	83.2 ± 0.25 a
Pectin	70.3 ± 1.30 a	7.71 ± 1.45 b	6.69 ± 0.46 c
Lignin	0.01 ± 0.01 c	0.06 ± 0.01 b	0.19 ± 0.04 a

Appendix V: Reagents and preparation of solutions.

Table I: Reagents.

Reagents	Mark	Country manufacturer
1,1-diphenyl-2-picrylhydrazyl (DPPH)	Sigma–Aldrich	Germany
sodium carbonate	Sigma–Aldrich	Germany
gallic acid	Sigma–Aldrich	Germany
Folin–Ciocalteu	Sigma–Aldrich	Germany

Table II: Preparation of solutions

Solutions	Reagents
Gallic acid	100 mg of Gallic acid + 10 mL of ethanol (30%)
Sodium carbonate	12 g of Sodium carbonate + 200 mL of distilled water
Solution of DPPH	12 mg of DPPH + 200 mL of ethanol + 100 mL of distilled water

Appendix VI: Calibration curve of phenolic compounds.

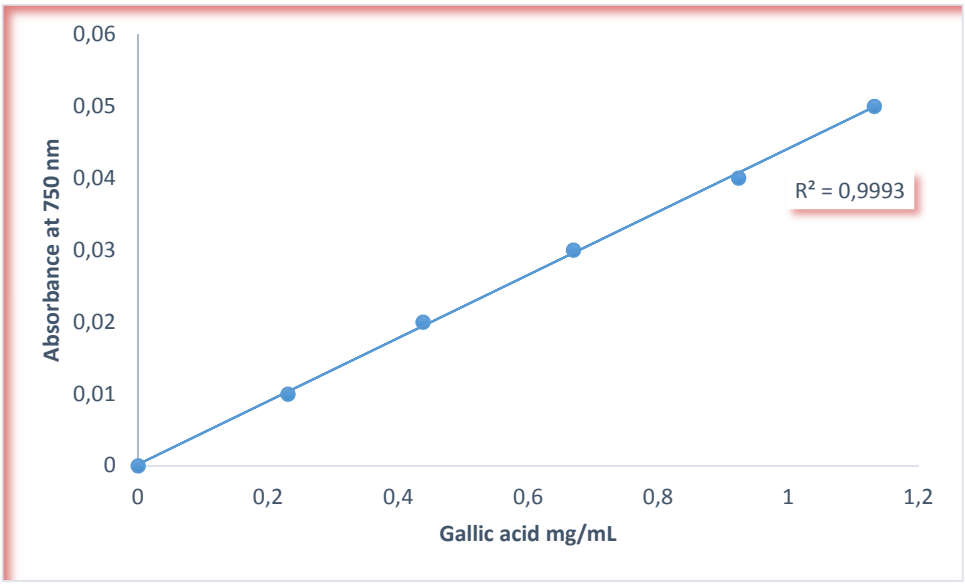

Figure I: Calibration curve of phenolic compounds

Abstract

This study reports the optimization of the conditions of extraction of TPC, Betalains and antioxidant activity from skin of prickly pear (*O. ficus-indica*) by ultrasound. Four independent variables, amplitude (20, 60, 100 %), extraction time (10, 20, 30 min), ethanol concentration (0, 30, 60 %) and liquid to solid ratio (20, 40, 60 mL) were studied.
The results showed that the highest TPC yield (1702mg GAE/100g DW) was obtained with an amplitude of 57%, extraction time of 17 min, ethanol concentration of 0% and liquid to solid ratio of 15 mL/mg. The highest yield of Betalains (61 mg/100g DW) was obtained with an amplitude of 63%, extraction time of 15min, ethanol concentration of 60% and liquid to solid ratio of 27 mL/g. Ultrasound assisted extraction is more efficient than conventional extraction method to obtain TPC, Betalains and antioxidant activity from skin of prickly pear. The experimental values were reasonably close to the predicted values confirming the validity of the predicted models.

Keywords: Skins from *Opuntia ficus indica*, Ultrasound assisted extraction, Total phenolic compounds, Betalains, Antioxidant activity, Response surface methodology (RSM).

Résumé

Cette étude rapporte sur l'optimisation des conditions d'extraction des composés phenoliques totaux (CPT), bétalaïnes et l'activité antioxydante des pelures de figue de barbarie (O. ficus-indica) par ultrasons. Quatres variables indépendantes, l'amplitude (20, 60, 100%), le temps d'extraction (10, 20, 30 min), la concentration en éthanol (0, 30, 60%) et le rapport liquide solide (20, 40, 60 ml) ont été étudiées.
Les résultats ont montrés que le rendement des composés phénoliques totaux le plus élevé (1702 mg GAE / 100 g DW) a été obtenu avec une amplitude de 57%, le temps d'extraction de 17 min, la concentration d'éthanol de 0% et un rapport liquide solide de 15 ml / mg. Le rendement le plus élevé des Bétalaïnes (61 mg / 100 g DW) a été obtenu avec une amplitude de 63%, le temps d'extraction de 15 min, la concentration en éthanol de 60% et un rapport liquide solide de 27 ml / g. L'extraction assistée par ultrason est plus efficace que les méthodes d'extraction conventionnelles pour obtenir les CPT, Bétalaïnes et l'activité antioxydante des pelures de figue de barbarie. Les valeurs expérimentales été très proche des valeurs prédites ce qui confirme la validité du modèle mathématique.

Mots-clés : Pelures d'*Opuntia ficus indica*, Extraction assistée par ultrason, Composés phénoliques totaux, Bétalaïnes, Activité antioxydante, Méthodologie des surfaces de réponses (MSR).