Optimization The Effect of Roasting Conditions by Central Composite Design (CCD) Method on The Antioxidant Compounds of *Opuntia Ficus Indica* Seeds From Morocco

Chakir El Guezzane
Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University, P.O. Box 1014 Agdal-Rabat, Morocco

Hamza El Moudden
Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University, P.O. Box 1014 Agdal-Rabat, Morocco

Hicham Harhar
Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University, P.O. Box 1014 Agdal-Rabat, Morocco

Abdelkader Zarrouk (azarrouk@gmail.com)
Universite Mohammed V de Rabat

Mohamed Tabyaoui
Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University, P.O. Box 1014 Agdal-Rabat, Morocco

Research

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Abstract

The effect of roasting conditions on antioxidant activity *Opuntia Ficus Indica* seeds from Morocco and their phenolic compounds were optimized by the Central Composite Design (CDD) method. The CCD was used to optimize the roasting conditions: temperature (X_1: 60–200 °C) and roasting time (X_2: 10–50 min). The best roasting conditions were used in order to optimize the optimal value of the response : TPC (Total Phenolic Content): X1: 200°C, X2: 50min with 104.86±1.94GAE/g extract predicted response, TFC (Total Flavonoids Content) : X1: 200 °C, X2: 50 min with 81.23±0.90 mg QE/g extract predicted responses, TTC (Total Condensed Tannins Content): X1: 128.9°C, X2: 34.92min with 6.12±0.95 mg QAE/g extract) predicted response. Moreover, the optimal potential antioxidant activity by ABTS assay and DPPH assay was found in extracts of *Opuntia Ficus Indica* seeds roasted: at a high temperature of the roasting. Furthermore, the positive significant correlations were determined by the principal component analysis (PCA) on the one hand, between the antioxidant capacity of the extracts and their antioxidants compounds (TPC and TFC), and on the other hand, between the two assays ABTS and DPPH. Consequently, the results of this work indicated that roasted *Opuntia Ficus Indica* seeds from Morocco can be considered as an essential ingredient for human foods.

Introduction

The Opuntia cactus is specie originally from Mexico (Mulas, 1992) and considered an important food for the indigenous populations (Barbera, Inglese, & Pimienta-Barrios, 1995). It is a specie of the Cactaceae family, the it is distributed in all continents as well as cultivated especially in the arid and semi-arid regions such as Africa and the Mediterranean region, South and Central America (Benayad et al., 2014). Moreover, the prickly pear fruit are rich in sugar, vitamin C, minerals, antioxidant compounds, and pigments, consequently, it was recommended in the human diet (Di Cagno et al., 2016). Furthermore, the seeds from Opuntia sp are considered rich in nutritional value, such as polyphenol, tannins, flavonoids, and fatty acids, in addition to that, the doses of these compounds are higher than in the pulp of the fruit, these fruits are eaten fresh with their seeds (Al Juhaimi et al., 2018). Also, the nutritional value and the chemical compounds of the oils of the seeds can be modified by roasting, this method is considered as a pretreatment which is done before extraction (Gao et al., 2019), and it can render the color, texture, and acceptability of grilled products (Khan & Saini, 2016). In addition to that, various studies showed that roasting pretreatment modifies the phenolic profile of the seeds; it can improve the health benefit effects (Carciochi, Galván D' Alessandro, Manrique, & technology, 2016). Consequently, the roasting can influence the antioxidant power of the seeds, this influence can depend on the formation of Maillard reaction products (MRP) and the thermal degradation of natural antioxidant molecules (Açar, Gökmen, Pellegrini, Fogliano, & Technology, 2009; Zhang, He, Hu, & technologies, 2011). Also, the study done by Sharma et (Sharma et al., 2015) showed that an increase in phenolic compounds and potential antioxidant capacity of six varieties of onions at 80° C,100°C,and 120°C. In this work, we are interested in the response surface methodology (RMS), it is a statistical technique useful for optimization. It is based on the most important variables and their effects of an experiment to build an empirical model, among the advantages of this

technique; it makes to reduce the number of experimental tests necessary to evaluate several factors and their interactions (Zhang et al., 2011). In the present work, we want to apply the Central Composite Design (CDD) approach, in order to investigate the effect of roasting treatment: roasting temperature, roasting time to maximize the contents of antioxidants compounds, and the antioxidant activity of *opuntia ficus idica* seeds. The main objective of this paper, we applied the CDD approach, in order to assess the effect of roasting treatment : roasting temperature and roasting time for to maximize the TPC(Total Phenolic Content),TFC (Total Flavonoids Content),TTC(Total Condensed Tannins Content), and the antioxidant properties by ABTS and DPPH assays for *opuntia ficus indica* from Morocco, Significant correlations between the antioxidant compounds and the antioxidant activities of *Opuntia ficus-Indica* seeds were evaluated by principal component analysis (PCA).

Materials And Methods

Plant materials

The plant material (Opuntia *ficus-indica seeds*) studied, were collected in the period between June and July 2019, in the region of Taza located in the East-North of Morocco.

Preparations of extracts

After the harvested of the fruits, the seeds were isolated, and then dried in the dark at room temperature for 72 hours, and they have been placed in an aluminum Petri dish (7 cm diameter) and roasted in a forced hot-air convection oven at 60, 130 and 200 °C for 10, 30 and 50 min. After that, the seeds will be crushed using a grinder, this fine powder will then be used for the preparation of the various extracts. Moreover, 40 grams of fine powder was macerated with the ethanol solvent for 48 hours. After that, the solvent was evaporated using a rotary evaporator. The extracts obtained are preserved at a temperature of about -4 ° C until the subsequent analyzes.

Chemicals and reagents

the chemical reagents that are used in these studies are categorized as follows : 2,2-diphenyl-1-pycridazil (DPPH. 90%) ,2,2'-azino-bis(3-ethylbenzthiazoline–6-sulphonic acid) (ABTS), Ascorbic acid, 2-thiobarbituric acid (TBA), aluminium chloride ($AlCl_3$), K2HPO_4, KH_2PO_4, ethylene-diaminetetraacetic acid (EDTA), Sulfuric acid and phenol and potassium persulphate,Folin–Ciocalteu's phenol reagent, aluminum chloride, , sodium acetate, sodium carbonate, sodium hydroxide , methanol, ethanol,ferrous sulfate, ferric chloride,) and 2,4,6-Tris(1-pyridyl)-5-triazine (TPTZ).

Determination of Total Phenolic Content (TPC)

The TPC of these extracts seeds was quantified by the method of singleton (Singleton, Orthofer, & Lamuela-Raventós, 1999). 200 µl of OFI seed extracts, add a volume of 1.5 ml of Folin Ciocalteu reagent (diluted 10 times). After 4 minutes, a volume of 1.5 ml of 5% sodium carbonate (Na_2 CO_3) was added on

to the solution. The tubes were placed in darkness. After two hours, Gallic acid was used as a standard for the calibration curve. The results were read on a spectrophotometer at 750 nm. The concentration of total polyphenols is deduced according to a calibration interval established with Gallic acid (0-100 µg / ml), as well as are presented in milligrams equivalent of a Gallic acid gram of extract (mg EGA / g extract).

Determination of Total Flavonoids Content (TFC)

The TFC was determined according to the method discussed by yeddes et al (Yeddes, Chérif, Guyot, Sotin, & Ayadi, 2013). 1 ml of extract is added to 1 ml of a solution of $AlCl_3$ (2% in methanol). After ten minutes of reaction, the absorbance is read at 430 nm. The flavonoid content is determined using a linear regression equation deduced from the calibration curve and expressed in milligrams equivalent of Quercetin per gram of extract (mg EQ / g extract). The flavonoid concentration is deduced from a calibration range established with Quercetin (0-100 µg / ml).

Determination of Total Condensed Tannins Content (TTC)

The TTC was done by the method described by the method described by sun et al (Sun, Ricardo-da-Silva, Spranger, & chemistry, 1998).50 mL of extracts seeds (50-600 mg / mL) was added to 3 mL of 4% methanolic vanillin solution and 1.5 mL of H_2SO_4. The absorbance was read at 430 nm after 15 minutes. The Catechin calibration curve was in the range of 50-600 mg / mL. TTC was presented as mg Catechin equivalent (CE) per gram of extract

Radical scavenging activity of DPPH1(1-diphenyl picrylhydrazyl)

The free radical removal capacity of the extracts was evaluated by the method of Grzegorczyk et al (Grzegorczyk, Matkowski, & Wysokińska, 2007). One ml for each extract at different concentrations (50 to 1000µg / ml) was mixed with one ml of a methanolic solution of DPPH at 0.1 mM and let sit for 30 min at 27 ° C. Methanol and DPPH were used as controls. After incubation at 37 ° C in the dark for 20 min, the absorbance was read at 517 nm. The antiradical capacity was quantified according to the following equation: % Radical scavenging activity DPPH = 1-[A_{sample} / $A_{control}$] × 100, where A sample and A control were the absorbance of the sample and control. The results obtained for each extract tested are compared with those obtained for ascorbic acid taken as standard antioxidant.

Radical cation inhibition activity (ABTS)

The radical cation capacity of OFI seed extracts was determined by the method described by Yim el al **(Yim et al., 2013)**. 88µL of 140mM of potassium persulfate ($K_2S_2O_8$) was added to 5ml of 7mM $ABTS^{\cdot+}$ solution. After that, the whole was stored in the dark at room temperature for 16h.Then,the absorbance of the $ABTS^{\cdot+}$ mixture was adjusted by ethanol to 0.70 ±0.05 at 734nm.10µ of OFI seed extracts at different concentration was mixed with 1 ml of ABTS reagent (100 to 1000µg / ml).The absorbance was read against the blank reagent at 734nm.The inhibition capacity was quantified uation according to the

following equation:: % Radical inhibition activity ABTS= 1- [$A_{extract}$ / $A_{control}$] × 100, where $A_{extract}$ and $A_{control}$ were the absorbance of the extract and control

Experimental design

In this study we used the CDD method, this method consists of 11 experimental assays (Table2) was employed for the optimization of roasting variables. The independent variables include roasting temperature and roasting time. These variable had a three levels (-1.0.+1) which are lower, medium, and higher (Table 1). TPC, TFC and TTC and antioxidant activity by ABTS assay and DPPH assay were selected such as the response of model design(Y) in this study; they are presented in Table 2. The regression coefficients (β) were obtained by the adjustment the experimental results to a second order polynomial model; this model was used to response analysis surface as below.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \qquad \text{(Eq 1)}$$

Where, Y is the response variable, X_i and X_j are the independent variables. β_0, $\beta_{i,}$ β_{ii} ,and β_{ij} are the regression coefficients of the model which are the constant, linear, quadratic ;and interactive effect respectively. These coefficients were obtained by analysis of variance ANOVA using JMP11 (SW) software. The p-value of the model, coefficient of determination (R^2), coefficient of adjustment (R^2_{adj}), and the p-value of lack of fit were used to the estimation of the adequacy of the polynomial equation to the responses. The desirability function was employed for multi-response optimization (Los, Zielinski, Wojeicchowski, Nogueira, & Demiate, 2019). All terms of the model were significant when their p-value is less than 0.05.3D response surface graphs were generated by JMP11(SW) software (Tomšik et al., 2016).

Table 1
Levels of variables of the roasting condition by CDD

Extraction process	Independent variables	Level		
		(-1)	(0)	(+ 1)
Maceration process	X_1 roasting temperature(°C)	60	130	200
	X_2 roasting time(min)	10	30	50

Data Analysis of principal component analysis (PCA) and matrix correlation

The correlations between all responses studied (TPC, TFC, TTC, ABTS, and DPPH) were determined by PCA. Also, PCA has represented the eleven extracts studied with the responses in a graph for facilitate comprehension the variation of the results of values photochemical responses according to the nature of the roasting condition.

Data Analysis

Central Composite Design (CCD) was done to the optimization of roasting conditions for *Opuntia Ficus Indica* seeds using, it was analyzed by JMP 11(SW) software. Moreover, the XLSTAT 2014 software (Zielinski et al., 2014) was used to determine the Pearson correlation between all data responses and for a graphical representation (PCA). Software IBM SPSS Statistics 21 was used to express the data in means ± standard error of the mean. The signification of data was done by the Tukey test at alpha = 0.05.

Results And Discussion

Table 2 present the results of the responses TPC, TFC, and TTC, DPPH, and ABTS of extracts from *opuntia ficus indica* seeds .This optimization of the roasting conditions was achieved in eleven randomized trials in order to evaluate the effects of different roasting factures on the studied responses.

Table 2
Experimental design and results of TPC, TFC, TTC, and antioxidant activity by DPPH assay, ABTS assay from opunitia ficus indica seeds extracts

Run	X_1	X_2	TPC	TFC	TTC	DPPH IC50	ABTS IC50
1	130	30	23.43 ± 1.23^{a}	53.71 ± 0.90^{ah}	6.8 ± 0.20^{a}	217.34 ± 2.96^{a}	410.62 ± 4.70^{a}
2	200	10	46 ± 0.56^{b}	49.14 ± 1.04^{h}	1.2 ± 0.30^{b}	149.72 ± 2.89^{b}	287.76 ± 2.09^{b}
3	130	50	41.85 ± 1.05^{b}	70.21 ± 0.79^{b}	4.6 ± 0.70^{ae}	144.10 ± 3.03^{b}	320.83 ± 4.93^{c}
4	130	30	2427 ± 1.20^{a}	57.21 ± 1.20^{a}	6.4 ± 0.95^{a}	212.93 ± 4.03^{a}	402.92 ± 3.98^{a}
5	200	30	59.86 ± 0.94^{d}	56.34 ± 0.96^{a}	1 ± 0.17^{b}	120.30 ± 2.18^{d}	240.013 ± 3.997^{d}
6	200	50	104.86 ± 1.94^{e}	81.23 ± 0.90^{c}	1.4 ± 0.23^{b}	90.663 ± 2.093^{e}	124.9 ± 4^{e}
7	60	10	21.57 ± 1.07^{af}	28.86 ± 1.10^{d}	0.4 ± 0.05^{b}	458.69 ± 3.70^{f}	624.736 ± 3.11^{f}
8	60	30	15.57 ± 1.10^{f}	30.43 ± 0.43^{de}	1.8 ± 0.33^{be}	375.92 ± 4.98^{g}	471.67 ± 2.90^{g}
9	130	10	20.71 ± 0.71^{af}	34.43 ± 1.03^{e}	2.1 ± 0.30^{be}	290.47 ± 3.79^{h}	546.48 ± 3.21^{i}
10	60	50	15.42 ± 1.02^{f}	63.29 ± 0.90^{g}	2.2 ± 0.60^{be}	265.94 ± 4.02^{i}	367.54 ± 2.10^{j}
11	130	30	26.23 ± 1.20^{a}	57.89 ± 1.09^{a}	6.1 ± 0.90^{a}	215.92 ± 3.02^{a}	413.97 ± 4.02^{a}

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM), the means followed by similar letters exposing in the same colum are not different ($P < 0.05$). TPC (mgGAE/gextract) : TFC(mgQE/gextract) : TTC(mgQAE/gextract) : DPPH IC50 (µg/ml) : ABTS IC50 (µg/ml), X_1 roasting temperature(°C) : X_2 roasting time(min)

Interpretation of the response surface model of TPC

Second-order polynomial model

In this study, the TPC of extracts from *opuntia ficus indica* seeds varied from 15.42 ± 1.02 to 104.86 ± 1.94 mg GAE / g extract. According to the results of ANOVA for TPC (Table 3),the model was significant (p-value < 0.0001).The lack of fit was not significant (P-value = 0.0862),which showed that the model equation was adequate to predicting the value values of the response. Additionally, the R^2 value was 0.990054 and the adjusted determination coefficient (R_{adj}^2 = 0. 980107), showing that the model adequately showed the true combination between all factors studied. According to Li et al (LI et al., 2019), when the determination coefficient was more than 0.75, the model is adequate. The equation Eq. 2 was represented according to a reduced regression model, it can predicate to the effects of factor variables on the content of TPC.

$$TPC = 24.093684 + 26.36X_1 + 12.308333X_2 + 16.2525X_{1*}X_2 + 14.445789X_{1*}X_1 + 8.0107895X_{2*}X_2 \quad \text{(Eq. 2)}$$

Table 3
Anova data of the regression coefficient and the terms of the model

Source	Coef	Sum of square	Degree of freedom	Mean square	F-value	p-value
TPC						
Model		7047.1123	5	1409.42	99.5379	< 0.0001*
Constant	24.093684					< 0.0001*
X_1	26.36	4169 .0976	1	4169 .0976	294.4350	< 0.0001*
X_2	12.308333	908.9704	1	908.9704	64.1944	0.0005*
$X_1 * X_2$	16.2525	1056.5750	1	1056.5750	74.6187	0.0003*
$X_1 * X_1$	14.445789	528.6581	1	528.6581	37.3355	0.0017*
$X_2 * X_2$	8.0107895	162.5710	1	162.5710	11.4813	0.0195*
Residual		70.7983	5	14.16		
Lack of fit		66.669210	3	22.2231	10.7642	0.0862
Pure Error		4.129067	2	2.0645		
Total Error		70.798277	5			
R^2		0.990054				
$Radj^2$		0.980107				
* Significant at p-value < 0.05						

X_1 had a significant positive linear effect (p-value < 0.0001), as well as, it's the quadratic X1* X_1 had a significant positive effect (p-value < 0.05) on TPC. Moreover, the linear effect of the roasting time X_2 and its quadratics effects X_{2*} X_2 were shown to have a significant positive effect respectively (p-value < 0.05).Furthermore, the interaction effect between the two parameters studied X_1* X_2 had significant (p-value < 0.05) on TPC.

Response Surface Methodology (RSM) analysis

The effects of both the roasting temperature and the roasting time and their reciprocal interactions on TPC can be visualized on the generating 3D response surface plots shown in Fig. 1. According to Fig. 1,

the TPC content increased when X_1 roasting temperature (°C) increased at a roasting time fixed, also it increased rapidly when the X2 roasting time (min)exceeds 30 min. Thus, the maximal extraction of TPC was found at the strong levels of both the roasting temperature (X_1) and roasting time (X_2). However, to get optimization overall of all variables studied. Optimization of the response was used by desirability function (d) in order to obtain the maximum response in TPC of the *opuntia ficus indica* seeds, thereby the maximum response precision is obtained when the desirability close to 1 (Figure.6) (Gullian Klanian & Terrats Preciat, 2017; Laib & Barkat, 2018; Los et al., 2019). Therefore, the optimal conditions were determined by using the JMP prediction profiler. The results regarding the optimized conditions of roasting by maceration extraction were when the desirability values (d = 0.89) close to 1 (Fig. 6.a): 200 °C, 50 min and predicted response is 101.4711 mg GAE/g extract). The experimental value was 104.86 ± 1.94 mg GAE/g extract, Therefore, the experimental and predicted responses were close. Hence, these results suggest that the model may be valid for the prevision of phenolic content by extraction maceration of the OFI seeds roasted. These results confirm the results found by several studies. Chandrasekara et al **(Chandrasekara, Shahidi, & Chemistry, 2011)** showed that the roasting at high temperature (130 °C) for 33 min had increased the phenolic content relative to raw seed (testa, cashew nuts).Yu et al (Yu, Ahmedna, & Goktepe, 2005) have also found that the TPC (in both water and ethanol) from peanut skin was increased about 35.9% by roasting at 175 °C for 5 min relative to the raw sample. Also, Locatelli et al (Locatelli et al., 2010) and Yin el al (Yin et al., 2019) respectively indicated that the TPC from extract soluble of hazelnut skin increased at 180 °C for 20 min more than 10 min,and the TPC increased about 3.4 times at temperature between 120 °C and 140 °C for 180 min, as well as, it increases more at 140 °C. Moreover, Kim et al (Kim et al., 2006) showed that, the TPC significantly increased (p-value < 0.05) at heat treatment. Accordingly, the increase in the content of phenolic compounds can be explained by the following causes:

During the roasting ,the molecules phenolic can be degraded/polymerized, which indicated the training of the new compounds, these compounds can be more soluble in ethanol and water, as well as, they can reagent with the Folin-Ciocalteu in alkaline middle(Yu et al., 2005).

It could be due processed by roasting, because the bound molecules bioactive can be released (Jeong et al., 2004).

Interpretation of the response surface model of TFC

Second-order polynomial model

Our results showed that, the TFC of extracts from *opuntia ficus indica* seeds varied from 28.86 ± 1.10 to 81.23 ± 0.90 mg QE / g extract. Moreover, ANOVA was used to verify the adequacy and the significance of the model. Table 4 showed that, the F-value is large (12. 9938) and the p-value is small (0.0069), which confirms that the model has been validated. Additionally, the lack of fit was not significant (p-value: 0. 0771) which indicates the was significant for TFC prediction of the *opuntia ficus indica* seeds roasted, the lack of fit cheks the inability of the model (LotfizadehDehkordi, Ghadimi, & Metselaar, 2013). The coefficients of determination R^2 and of adjusted had high values R^2 = 0.92854 and Radj2 = 0.85708 respectively. These values indicated that the quality of the model is valid. The equation which combines the relationship between the variables and prediction TFC was described below.

$$TFC = 52.860526 + 10.688333X_1 + 17.05X_2 - 0.585X_{1*}X_2 - 4.361316X_{1*}X_1 + 4.5736842X_{2*}X_2 \quad \text{(Eq. 3)}$$

Table 4
ANOVA data of the regression coefficient and the terms of the model.

Source	Coef	Sum of square	Degree of freedom	Mean square	F-value	p-value
TFC						
Model		2510.9388	5	502.188	12.9938	0.0069*
Constant	52.860526					< 0.0001*
X_1	10.68833	685.4428	1	685.4428	17.7354	0.0084*
X_2	17.05	1744.2150	1	1744.2150	45.1306	0.0011*
$X_1 * X_2$	-0.585	1.3689	1	1.3689	0.0354	0.8581
$X_1 * X_1$	-4.361316	48.1867	1	48.1867	1.2468	0.3149
$X_2 * X_2$	4.5736842	52.9938	1	52.9938	1.3712	0.2944
Residual		193.2410	5	38.648		
Lack of fit		183.17941	3	61.0598	12.1372	0.0771
Pure Error		10.06160	2	5.0308		
Total Error		193.24101	5			
R^2		0.92854				
$Radj^2$		0.85708				

* Significant at $p < 0.05$

Roasting time(X_2) had a positive significant linear effect (p-value = 0.0011 < 0.05) on TFC, and it doesn't have a significant quadratic effect (p-value = 0.2944).As well as, the Roasting temperature X1 had significant positive linear (p-value = 0.0084), but its quadratic effect is not significant (p-value = 0.2944). Moreover, the not significant interaction effect between the two parameters studied was observed (Table 4).

Response Surface Methodology (RSM) analysis

Figure 2 shows the response surface plot of roasting temperature and roasting time on total flavonoid content. The TFC increased with the increased both the roasting time and the roasting temperature. Accordingly, the higher TFC yield was detected in regions of high roasting temperature and hard roasting time. Consequently, the optimum extraction of TFC was at: roasting temperature 200°Cand roasting time 50 min, and it was assigned for the predicted response is 80.22623 (mg QE/g extract) with the desirability is d=0.84(Figure.6.b.). Our results are similar with various studies as, Lin et al **(Lin et al., 2016)** reported that the TFC increased significantly after 5min of the roasting, as well as, the flavonoid aglycones and acids are increased according to roasting temperature and time. Furthermore, Kumar et al **(Kumar & Pandey, 2013)** mentioned that, the fractions of sugar in flavonoids glycosides have an important role in antioxidants capacity, as well as, the aglcycones had a high effect on the antioxidant capacity more than the glycosides.

Interpretation of the response surface model of TTC

Second-order polynomial model

Table 5 shows the coefficients of regression and their significance for the TTC yield. The regression model was significant (p-value = 0.0231). Also, the determination coefficients (R^2) for the TTC response variable (0.881256) and the lack-of-fit values (0.0537) were not significant ($P > 0.05$), which indicates that the model can explain all data. So the response variable was included in roasting optimization. Besides, the R^2_{adjs} was 0.762512,it indicated that 76.25% of the variability was estimated by the model. Therefore, the second-order polynomial model was applied (Eq. 4).

$TTC = 5.8052632-0.133333X_1 0{,}75X_2-0{,}4X_1{*}X_2-3.463158X_1{*}X_1-1.513158X_2{*}X_2$(Eq. 4)

Table 5

ANOVA data of the regression coefficient and the terms of the model.

Source	Coef	Sum of square	Degree of freedom	Mean square	F-value	p-value
TTC						
Model		50.697863	5	10.1396	7.4215	0.0231*
Constant	5.8052632					0.0002*
X_1	-0.133333	0.106667	1	0.106667	0.0781	0.7911
X_2	0.75	3.375000	1	3.375000	2.4703	0.1768
$X_1 * X_2$	-0.4	0.640000	1	0.640000	0.4684	0.5241
$X_1 * X_1$	-3.463158	30.383439	1	30.383439	22.2386	0.0053*
$X_2 * X_2$	-1.513158	5.800439	1	5.800439	4.2455	0.0944
Residual		6.831228	5	1.3662		
Lack of fit		6.5845614	3	2.19485	17.7961	0.0537
Pure Error		0.2466667	2	0.12333		
Total Error		6.8312281	5			
R^2		0.881256				
$Radj^2$		0.762512				

* Significant at $p < 0.05$

According to p-value < 0.05, the $X_1 * X_1$ is the quadratic effect of roasting temperature was positive significant for TTC, on the contrary, its linear effect did not have significant because of the p-value = 0.791. As well as, the X2 and $X2 * X_2$ of roasting time were not had significant in TTC because, their p-value was respectively: 0.1768, 0.0944. The $X_1 * X_2$ had also not significant according to its p-value was 0.5241.

Response Surface Methodology (RSM) analysis

The 3D of response surface of regression Eq. (3) were constructed using RSM to illustrate the effects of the roasting temperature and roasting time and their interaction on the TTC (Fig. 3). Accordingly, the TTC content increased before the roasting temperature increased at 130 °C, after that it decreased quickly. Also, the TTC increased with the roasting time in the range of 10 to 35 min and then decreased.. The optimum extraction of TTC was roasting temperature 128.9 °C, roasting time 34.92 min with 5.901 (mg QAE/g extract) predicted responses and the desirability is d = 0.82(Figure.6.c.).These results are similar to these reported by Lin et al (Lin et al., 2016), they showed that, during the roasting at 200 °C for 20 min the content of condensed tannins from ethanol extracts had a high levels in TTC.

Interpretation of the response surface model of DPPH assay

Second-order polynomial model

The ANOVA results from DPPH assay content based on the RSM design are reported in Table 6.The p-value of the model was (< 0.0001),which indicated that the model was significant. Moreover, the R^2 and R^2_{adj} were 0.997921 and 0.995841 respectively, that confirms the adequacy of the model because $R^2 >$ 0.75 (LI et al., 2019). Additionally, the lack of fit (p-value > 0.05) confirms also the adequacy the model for prediction of the antioxidant power for *opuntia ficus indica* seeds roasted. Therefore, the second-order polynomial model was applied (Eq. 5).

$DPPH\ (IC_{50}) = 217.23774-123.3112X_1-66.36283X_{2+}33.42325X_1{*}X_2 + 28.110658X_1{*}X_1-2.714342\ X_2{*}X_2$ (Eq. 5)

Table 6
ANOVA data of the regression coefficient and the terms of the model.

Source	Coef	Sum of square	Degree of freedom	Mean of square	F-value	p-value
DPPH						
Model		124190.70	5	24838.1	479.9081	< 0.0001*
Constant	217.23774					< 0.0001*
X_1	-123.3112	91233.863	1	91233.863	1762.768	< 0.0001*
X_2	-66.36283	26424.154	1	26424.154	510.5521	< 0.0001*
$X_1 * X_2$	33.42325	4468.455	1	4468.455	86.3369	0.0002*
$X_1 * X_1$	28.110658	2001.863	1	2001.863	38.6788	0.0016*
$X_2 * X_2$	-2.714342	18.665	1	18.665	0.3606	0.5743
Residual		258.78	5	51.8		
Lack of fit		248.64531	3	82.8818	16.3558	0.0582
Pure Error		10.13487	2	5.0674		
Total Error		258.78018	5			
R^2		0.997921				
$Radj^2$		0.995841				

* Significant at $p < 0.05$

Based on statistical analyses of ANOVA for DPPH, the roasting temperature (X_1) and roasting time (X2) had a negative significant linear effect on the IC50 of DPPH assay, because their P-value is equal respectively a <0.0001*and <0.0001*.As well as, their interaction had significant for DPPH because the p-value was 0.0002*.On the contrary, their quadratic effects were not had significant due to their p-value was >0.005.

Response Surface Methodology (RSM) analysis

The response surface (3D) of regression Eq. (5) were constructed using RSM to illustrate the effects of X1 and X_2 and their interactionX_1*X_2 on the IC_{50} of DPPH assay (Fig. 4.). We know that the antioxidant power is inversely proportional with the value of the IC_{50}. We observed that the increase in the antioxidant power was made thanks to the increasing of the X_2 roasting time and the increasing of the roasting temperature (X_1). The optimum of the antioxidant power by DPPH assay observed at roasting temperature 200 °C and roasting time 50 min with 96.60% of inhibition, which matches 86.3845 µg/ml predicted responses, and also the desirability is d = 0.93(Figure.6.d.).Our results confirm those founded by Lin et al (Lin et al., 2016), they reported that, the higher antioxidants capacity was observed at strong roasting temperature for ethanol extracts from almond*(Prnus duclis)*kernel, and during the roasting at 200 °C for 20 min the power for scavenging DPPH radical was strong than the raw sample. Moreover, Chandrasekra et al (Chandrasekara et al., 2011), reported that in their study, the scavenging capacity of DPPH radical increased significantly with the increase of the roasting temperature for soluble phenolic extract from *testa*, as well as, they justified that increase due to Maillard reaction products MRPs. Indeed, during at roasting, a reaction between the reducing sugars and amino acids can be done, this reaction can produce the new compounds, which are Maillard reaction products MRPs, these formed products can contribute to TPC, flavour, antioxidant activity and color of food (Chandrasekara et al., 2011). Furthermore, the resultant melanoidin and the intermediate Maillard reaction products (MRPs) had strong antioxidant pwer,which are according to the presence of reductone-type structures (Hayase, Hirashima, Okamoto, Kato, & Chemistry, 1989).

Interpretation of the response surface model of ABTS assay

Second-order polynomial model

Experimental modeling results for antioxidant power by ABTS assay were shown in Table 7. From the model analysis, the R^2 and R^2_{adj} of the model were 0.99075, 0.9815 respectively, also did not present lack of fit (p-value = 0. 0528). Moreover, the model was significant because its p-value was < 0.0001,which showed that the model equation was acceptable to predict the antioxidant power by ABTS assay. this model equation is shown in Eq. 6 as follows:

ABTS (IC_{50}) =415.29474-135,2122X_1-107.6177X_{2+}23.584X_1*X_2-68.64034X_1*X_1+9.1731579X_2*X_2(Eq 6)

Table 7

ANOVA data of the regression coefficient and the terms of the model.

Source	Coef	Sum of square	Degree of freedom	Mean square	F-value	p-value
ABTS						
Model		193571.35	5	38714.3	107.1061	< 0.0001*
Constant	415.29474					< 0.0001*
X_1	-135.2122	109693.98	1	109693.98	303.4772	< 0.0001*
X_2	-107.6177	69489.37	1	69489.37	192.2479	< 0.0001*
$X_1 * X_2$	23.584	2224.82	1	2224.82	6.1551	0.0558
$X_1 * X_1$	-68.64034	11935.79	1	11935.79	33.0213	0.0022*
$X_2 * X_2$	9.1731579	213.17	1	213.17	0.5898	0.4772
Residual		1807.29	5	361.5		
Lack of fit		1743.0803	3	581.027	18.0991	0.0528
Pure Error		64.2050	2	31.103		
Total Error		1807.2853	5			
R^2		0.99075				
$Radj^2$		0.9815				
* Significant at p < 0.05						

Roasting time (X_1) and roasting temperature (X_2) had a negative significant linear effect (p < 0.05). Also, the quadratic effect of the roasting temperature X1 * X1 had significant effect (p-value = 0.0022), and the interaction effect between the two parameters roasting was not significant (Table 7).

Response Surface Methodology (RSM) analysis

Figure 5 shows the IC_{50} of ABTS assay, we observed that the antioxidant power increase significantly when the roasting temperature X_1 and roasting time X_2 increased, because the IC_{50} decreased. According to, the more IC_{50} decreases the more the antioxidant power increases. The optimum of the antioxidant power by ABTS assay was at: roasting temperature 200°C and roasting time 50 min with 96.75% of inhibition, which matches 130.581 µg/ml predicted responses, and also the desirability is d=0.89 (Figure.6.e.). These results are similar to several works as Gao et al **(Gao et al., 2019)** mentioned that, the ABTS capacity was increased significantly during the roasting at 160°C for 10min compared to raw sample. Also Yin et al **(Yin et al., 2019)** reported that the ABTS scavenging increased during the heating between 130°C-140°C after 60min.Moreover, these results can depend on several conditions such as, the plants have a bound antioxidant phenol and bound polymeric compounds, during the thermal treatment these molecules can be degraded and released which leads to an increase in the antioxidants activity **(Lee, Kim, Kim, Jang, & chemistry, 2002)**. Furthermore, after the antioxidants characteristics can be improved thanks to the degradation of the heat-labile antioxidants compounds or training the new

compounds by Maillard reaction **(Nicoli, Anese, Parpinel, & Technology, 1999)**. Also, the solubility of non-phenolic molecules was improved by roasting **(Dewanto, Wu, Adom, Liu, & chemistry, 2002)**.

Comparisons of predict (models) and experimental results.

The verification experiments for five responses such as antioxidants activity by DPPH IC_{50} (µg/ml), ABTS•+ inhibition activityIC_{50} (µg/ml), Total phenolic Contents (mg GAE/g extract, Total flavonoids (mg QE/g extract), and Total Tannins Content (mg QAE/g extract) were reported in Table 8. These experiments were done at the conditions of responses optimal and in the experimental range. these results showed that the values of responses experimental are close to those predicted.

Table 8: predicted and experimental results at conditions optimal

Total phenolic Contents (mg GAE/g extract)	X_1 roasting temperature (°C)	X_2 roasting time (min)	Predicted value	Experimental value
Total flavonoids content (mg QE/g extract)	200°C	50min	80.22623	81.23±0.90
Total Tannins Content (mg QAE/g extract)	128.9°C	34.92min	5.901	6.12±0.95
Total phenolic Contents (mg GAE/g extract)	200°C	50min	101.4711	104.86±1.94
DPPH IC50 (µg/ml)	200°C	50min	86.3845	90.663±2.093
ABTS IC50 (µg/ml)	200°C	50min	130.581	124.9±4

The data are presented in the form of the average of two individual repetitions (n = 2e ± SEM),

Correlation Matrix

Table 9 showed the correlations coefficients data between all responses studied. Moreover, Table 10 presents the p-value of these correlation coefficients. Additionally, the DPPH (1/DPPH IC_{50}) and ABTS (1/ABTS IC_{50}) represent the power to inhibit DPPH free radical and $ABTS.^+$ radical respectively.

Table 9

Pearson's correlation matrix coefficient between antioxidant capacity and antioxidants compounds in the extracts from *Opuntia Ficus Indica*

Variables	TPC	TFC	TTC	DPPH (1/DPPH IC_{50})	ABTS (1/ABTS IC_{50})
TPC	**1**				
TFC	**0.652**	**1**			
TTC	-0.301	0.287	**1**		
DPPH (1/DPPH IC_{50})	**0.949**	**0.784**	-0.121	**1**	
ABTS (1/ABTS IC_{50})	**0.966**	**0.727**	-0.255	**0.920**	**1**

The values is bold are different from 0 at a significance level alpha = 0.05.TPC (Total Phenolic Content), TTC :Total Condensed Tannins Content, TFC : Total Flavonoïdes Content, DPPH(1/DPPH IC_{50}): 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity;ABTS(1/ABTS IC_{50})

Table 10
p-values of the correlation matrix coefficient between all variables

Variables	TPC	TFC	TTC	DPPH (1/DPPH IC_{50})	ABTS (1/ABTS IC_{50})
TPC	**0**				
TFC	**0.030**	**0**			
TTC	0.369	0.392	**0**		
DPPH (1/DPPH IC_{50})	**< 0.0001**	**0.004**	0.723	**0**	
ABTS (1/ABTS IC_{50})	**< 0.0001**	**0.011**	0.449	**< 0.0001**	**0**

The values is bold are different from 0 at a significance level alpha = 0.05.

According to Table 9 and Table 10, we observed that, the TPC had high positive correlations significant (p-value < 0.05) between the antioxidants power. The correlation coefficients of TPC were 0.949 and 0.966 with free radical scavenging effect DPPH and $ABTS^{\cdot+}$ respectively. These positive correlations are justified that the antioxidants capacity depends on the presence of phenolic compounds in *opuntia ficus indica* seeds extracts,these results are similar with those reported by several study (**Amri et al., 2015; Cheniany et al., 2013; Guettaf, Abidli, Kariche, Bellebcir, & Bouriche, 2016**). It found that, the TFC had also the positive correlations significant between DPPH (1/DPPH IC_{50}) and ABTS (1/ABTS IC_{50}) , these correlation coefficients were 0.784 and 0.727 respectively. Therefore, these results are confirmed by the strong positive correlation significant (p-value < 0.05) between TFC and TFC (r^2 = 0.652). We observed that, the p-values of TTC with the antioxidants power were not significant (p-value > 0.05), which indicates that Tannin contribute slightly in this bioactivity. Furthermore, the strong positive correlation significant between tow antioxidant capacity (r^2 = 0.920),indicates that the same bioactive molecules in our extracts are responsible to the scavenging power of two free radicals DPPH and $ABTS^{\cdot+}$ ..

Principal Component Analysis (PCA).

According to Fig. 8, the projections of the responses studied and the experiment assays (extracts) were done by the factorial plan reported in Fig. 8.The cumulative percentage was 95.14%, which indicates that it was is representative of the variables because it was more the 50%. Moreover, the two axe are suitable for explains the all information, with the frits (F1) and second (F2) main components have explained 70.63% and 24.51 the information respectively. The correlations between all variables studied were explained by a plan formed by F1 and F2 axes. Besides, the F1 axe was formed by the positive correlation between TPC,TFC,ABTS($1/IC_{50}$),DPPH($1/IC_{50}$)),on the contrary the F2 axe was constructed by TTC (Fig. 7).Ours 11 extracts studied from *opuntia ficus indica* seeds, were distributed in three groups according the responses (Fig. 8).

Group I:this group was formed by four extracts(2,3,5,6) ,these extracts had a strong values of the TPC and TFC, as well as, they had also a high power antioxidants by DPPH and ABTS assays.

Group II: it contains four extracts (1, 4, 10, and 11), these extracts are characterized by a strong value of TTC, and by lower values of TPC and TTC. Therefore, their antioxidants activity is lower compared to group I.

Group III is formed by a three extract (7, 8 and 9), these extracts are characterized by the low values of TPC and TFC, and its antioxidant activity is low compared to extracts of the other groups.

The extracts from Group I are characterized by a high roasting temperature varies between 130 °C and 200 °C, and a high time of roasting (50 min) for the extracts roasted at 130 °C, which shows that their a strong antioxidants capacity more than the extracts from the Group II and III obtained by low roasting temperature. Therefore, the roasting makes it possible to increases the extraction of bioactive compounds responsible for antioxidants power.

Conclusion

Dry thermal processing of seeds from the *Opuntia ficus indica* increased the amounts of active compounds. As well as, antioxidant activity power was significantly improved in extracts roasted especially at stronger temperature. Furthermore, the results indicated that the temperature and time of roasting had significant effects.PCA showed that on one hand the positive correlation between the photochemical compounds (polyphenol, flavonoids) and the antioxidant capacity (ABTS, DPPH),Consequently, this study showed thermal processing can be used as a pre-treatment to increase the antioxidants capacity of *Opuntia ficus Indica* seeds.

Abbreviations

CDD : Central Composite Design; X_1: roasting temperature; X_2 : roasting time(min); TPC : Total Phenolic Content; TFC: Total Flavonoids Content; TTC : Total Condensed Tannins Content; PCA: Principal Component Analysis; MRP: Maillard Reaction Products; RMS: Response Surface Methodology; DPPH: 2,2-diphenyl-1-pycridazil;ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline–6-sulphonic acid; IC50: half-maximal inhibitory concentration; ANOVA: Analysis of Variance; Eq: equation; R^2 : coefficient of determination; R^2_{adj}: Coefficient of Adjustment; d: desirability function; r^2 : coefficient of correlation; F1:Frist main component; F2 :Second main component; GI: Group I; GII: Group II; GIIi : Group III.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

C-EG, H-H, A-Z, and M-T conceived and designed the experiments. C-EG and H-EM have carried out experiments. All authors discussed the results and co-wrote the manuscript. All authors read and approved the final paper.

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Figures

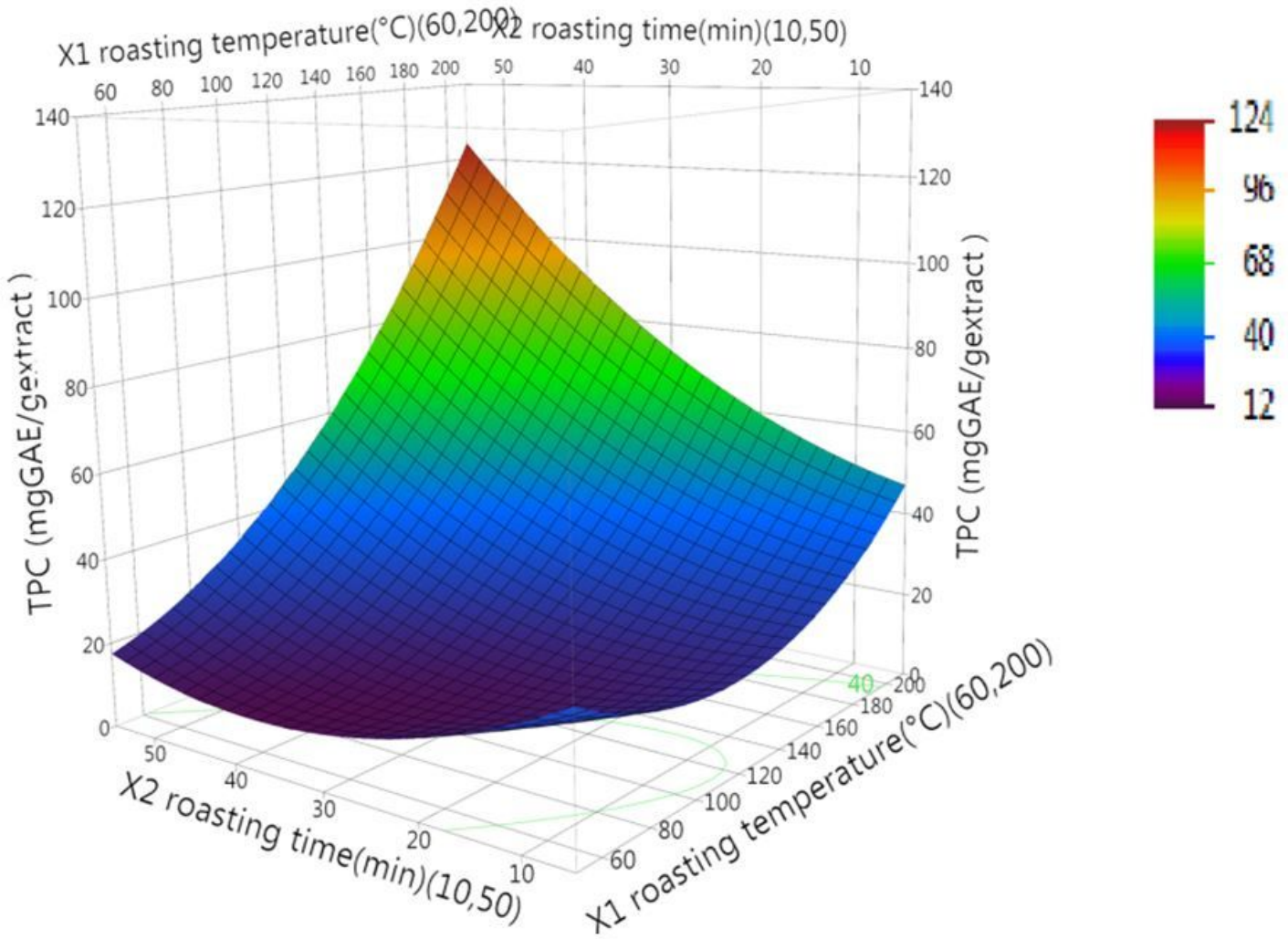

Figure 1

Response surface plots of roasting conditions of TPC of the opuntia ficus indica seeds extracts.

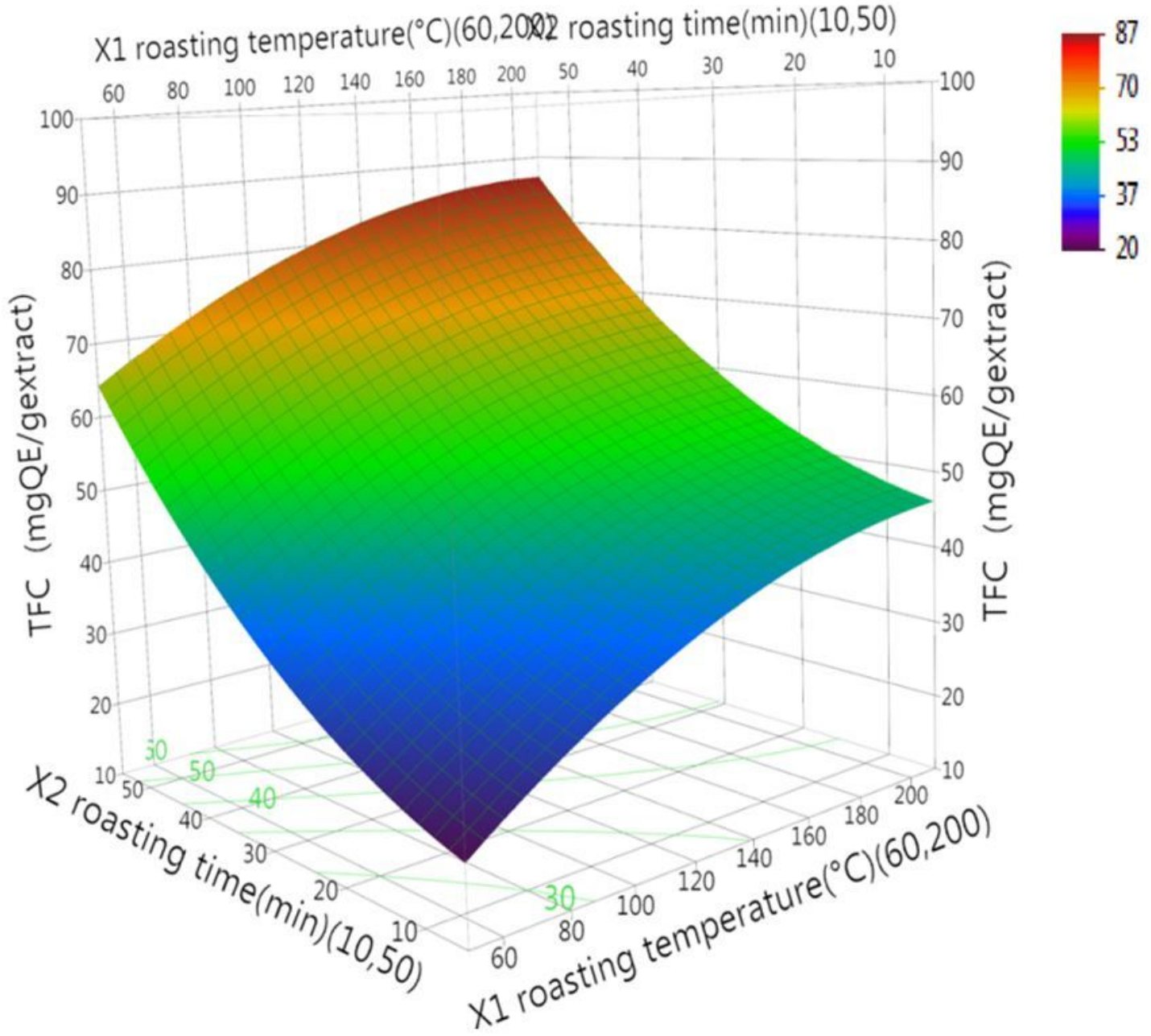

Figure 2

Response surface plots of roasting conditions of TFC of the opuntia ficus indica seeds extracts.

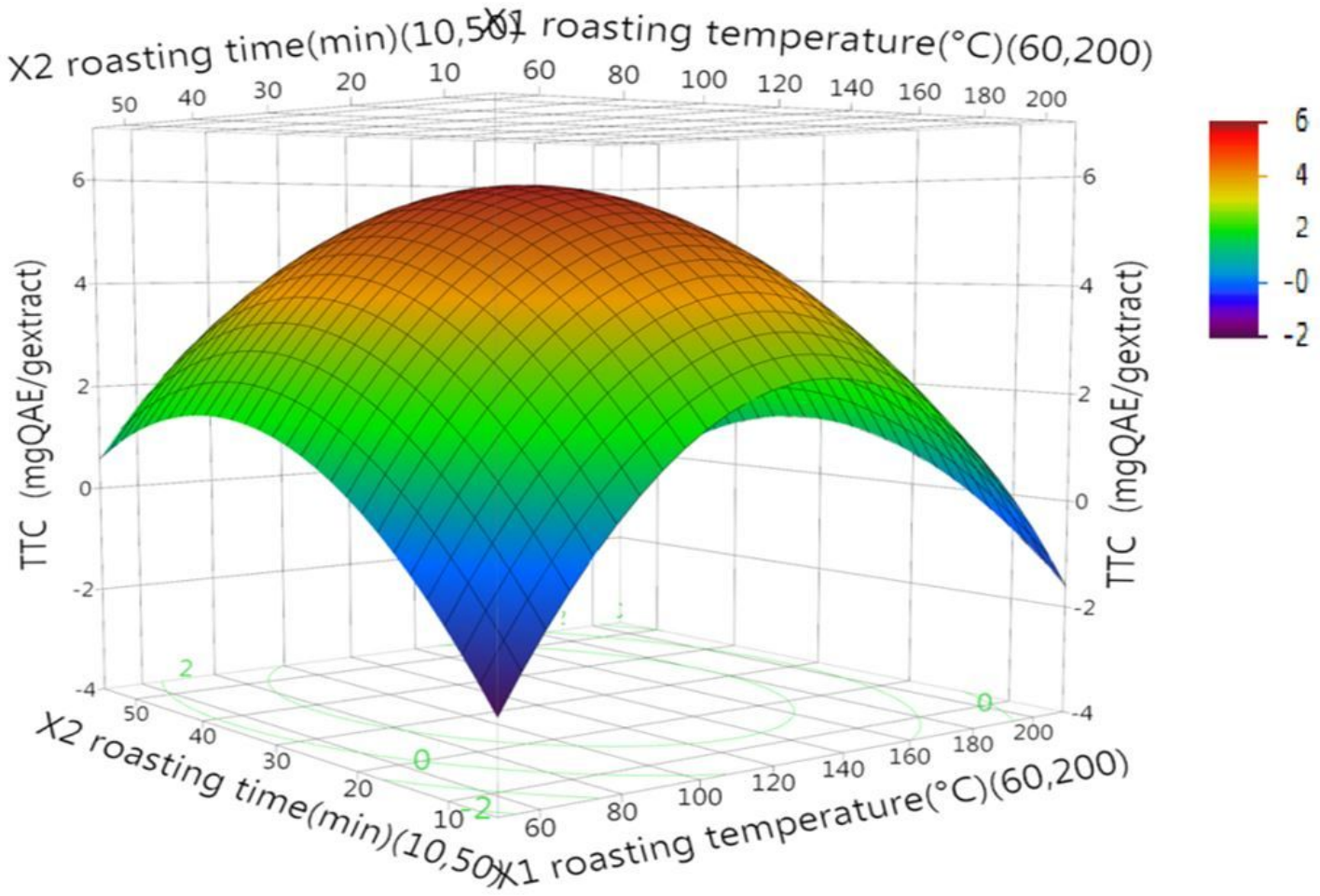

Figure 3

Response surface plots of roasting conditions of TFC of the opuntia ficus indica seeds extracts.

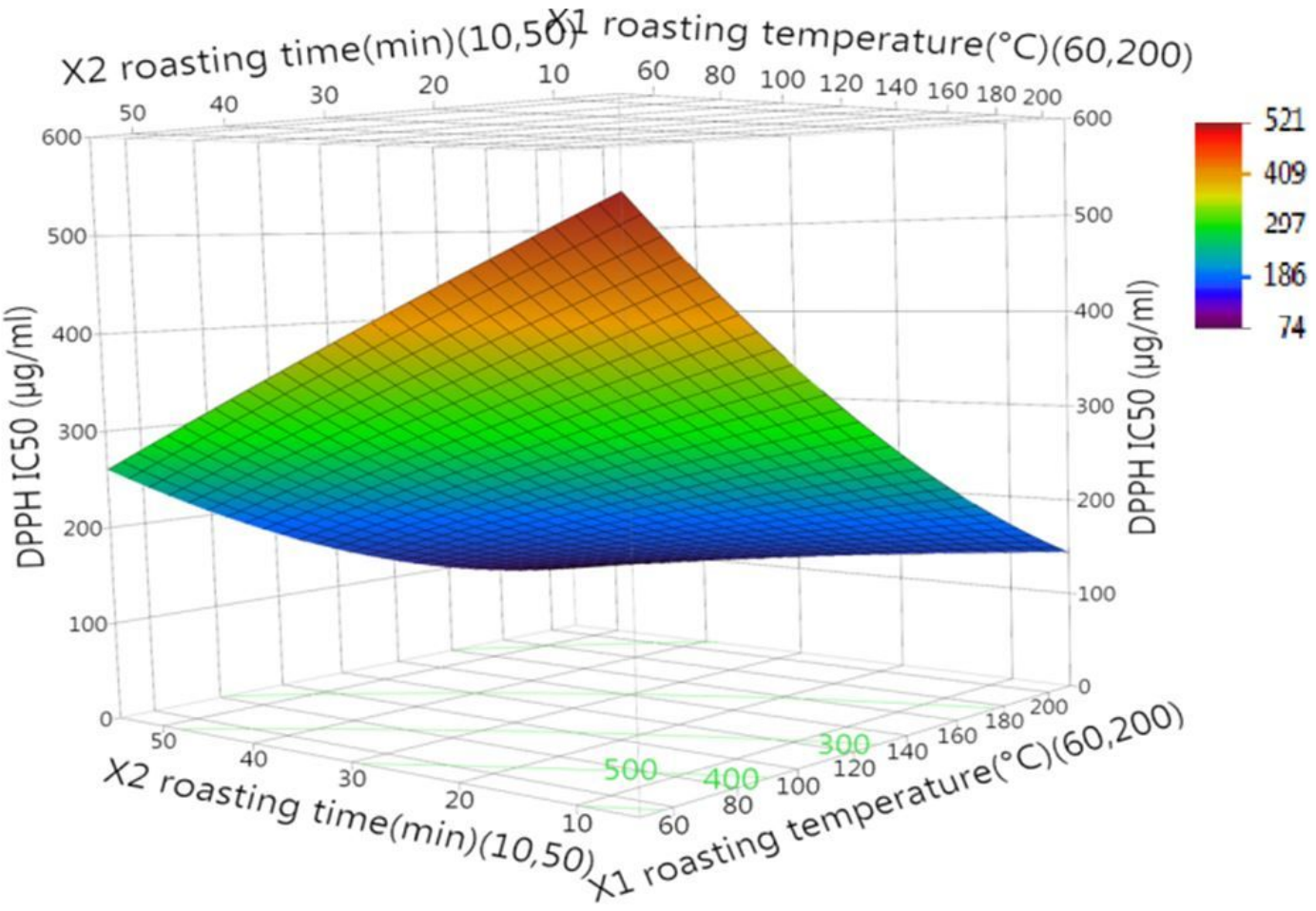

Figure 4

Response surface plots of roasting conditions of DPPH IC50 of the opuntia ficus indica seeds extracts.

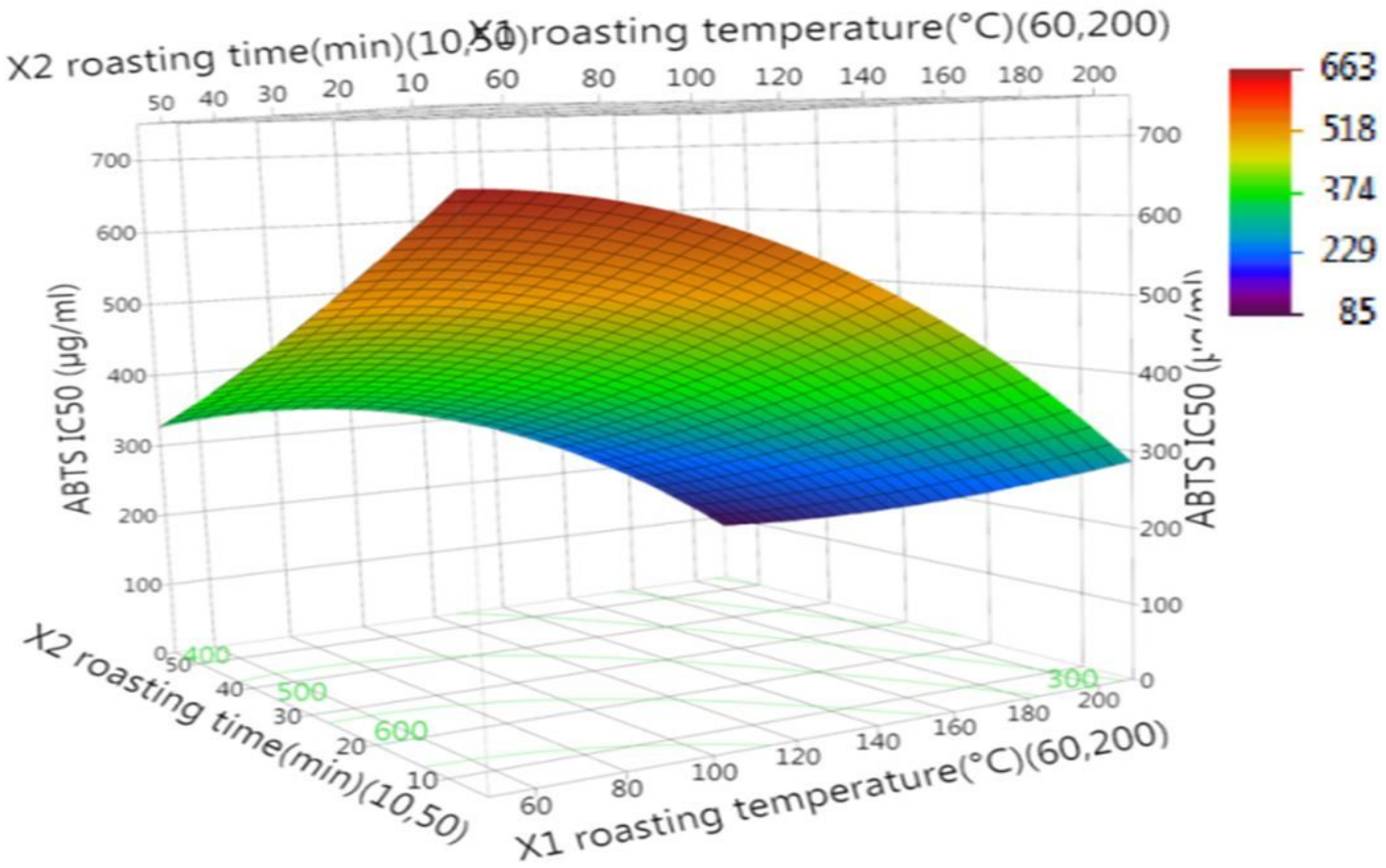

Figure 5

Response surface plots of roasting conditions of ABTS IC50 of the opuntia ficus indica seeds extracts.

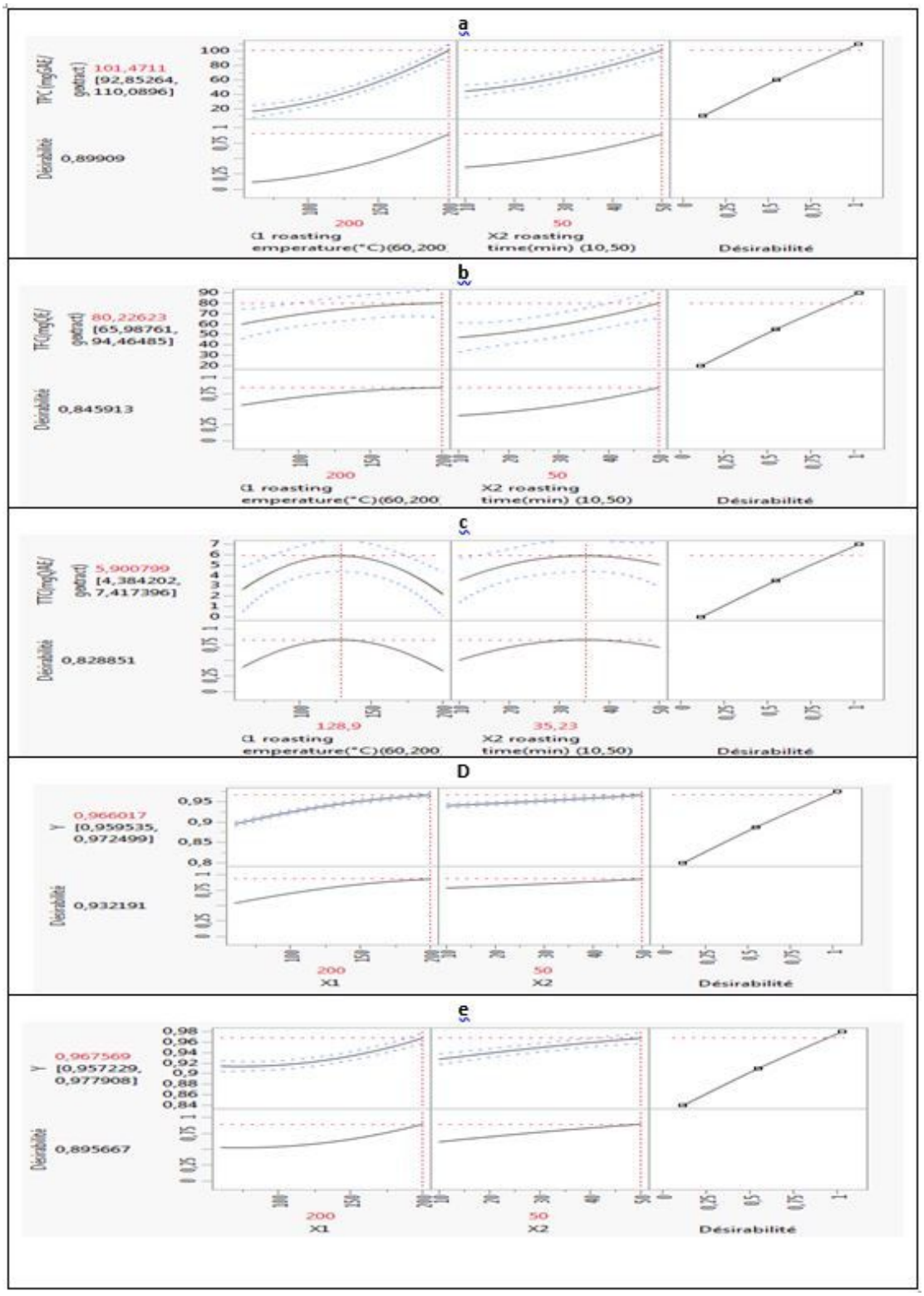

Figure 6

Desirability results for: a: TPC, b: TFC, c: TTC, d: DPPH, and e: ABTS.

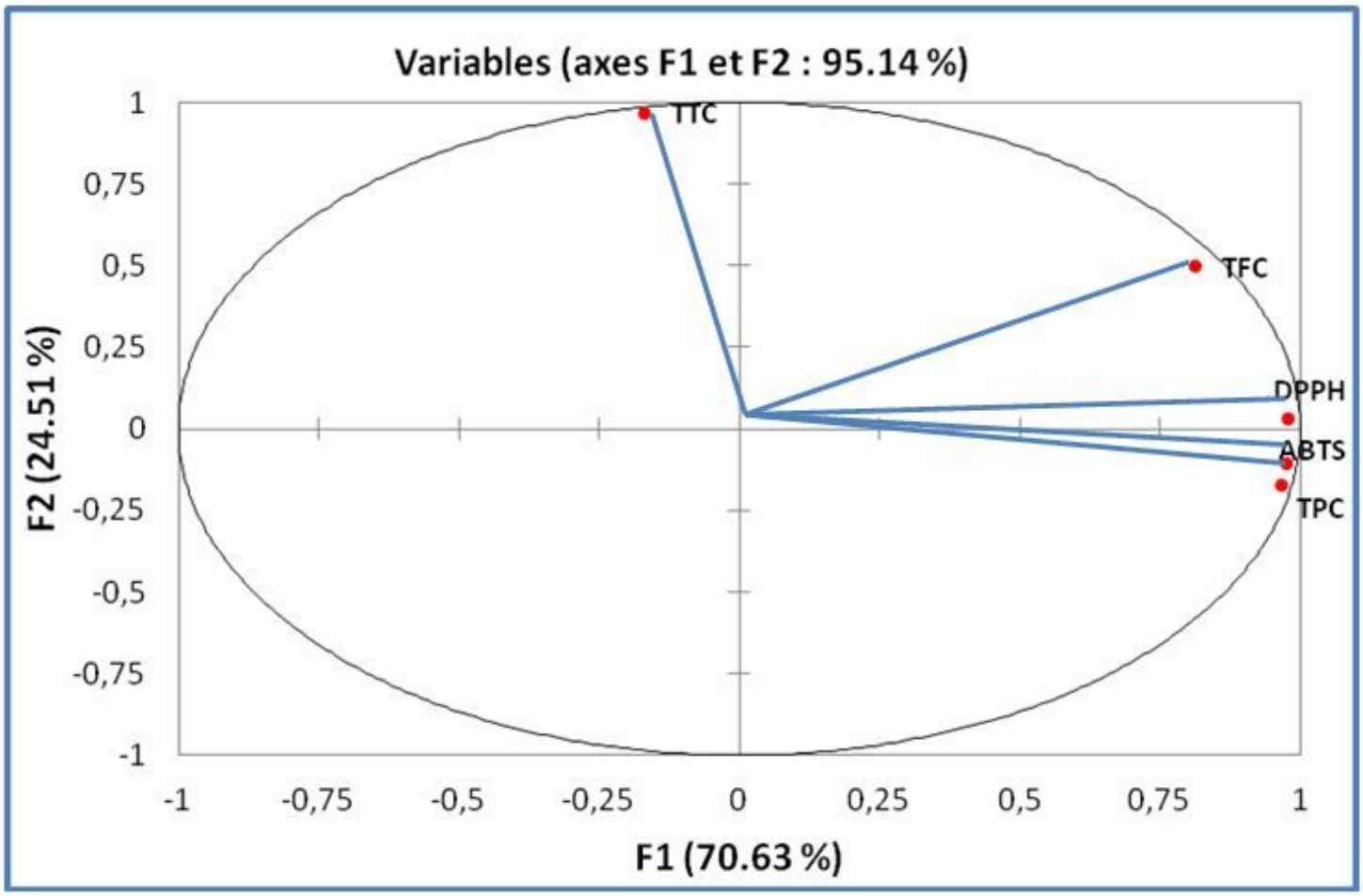

Figure 7

Principal Component Analysis factorial plan carried out on the values (TPC, TFC, TTC, DPPH and ABTS) of the different extracts from Opuntia Ficus Indica DPPH (1/DPPH IC50); ABTS (1/ABTS IC50)

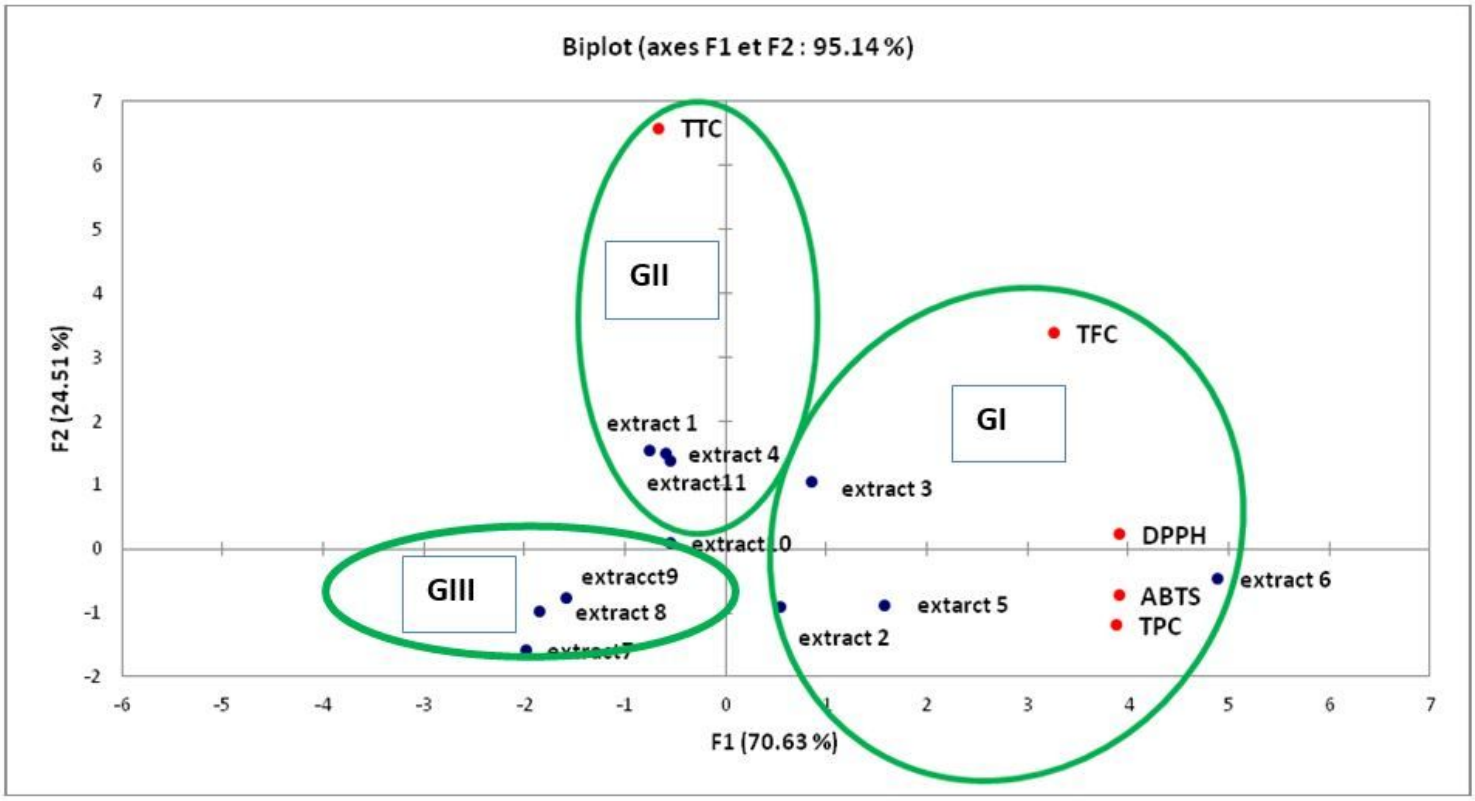

Figure 8

Projection on the factorial plan (F1×F2) of the individual's variable. DPPH (1/DPPH IC50); ABTS (1/ABTS IC50), GI: Group I; GII: Group II; GIIi ;Group III,

Supplementary Files

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- GraphicalAbstract.doc