Optimization of Roasting Conditions on the Bioactive Compounds and their Antioxidant Power from *Opuntia fusicus-Indica* Seeds using Response Surface Methodology (RSM)

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**Abstract:** This work is aimed to optimize the effects of different roasting factors on the bioactive compound’s antioxidant capabilities of *opuntia fusicus indica* seeds by using DPPH and ABTS assays. The Box–Behnken design (BBD) was applied to optimize the roasting temperature (X1:60-200 °C), roasting time (X2:10-50 min), and extraction time (X3: 3–6h). The optimal conditions of roasting applied in order to optimize the greatest content of the responses were: TPC (Total Phenolic Content): X1:200 °C, X2: 50 min, and X3: 5.49 h with 103.5±0.76 GAE/g extract experimental response, TFC (Total Flavonoids content): X1:200°C, X2:50min, and X3:3.16 h with 104.29±0.54 mg QE/g extract experimental response, TTC (Total Condensed Tannins Content): X1: 200°C, X2: 50min, X3:6h with 10,16±0.89 mg QAE/g extract experimental response, TSC (Total Sugars Content): X1: 60°C, X2:50 min, X3:5.12 h with 40.35±0.99 mg D-Glu E/g extract an experimental response. The maximal antioxidant power by DPPH and ABTS assays was recorded at a strong temperature of roasting. Moreover, the significant correlations were obtained by the principal component analysis (PCA), the first step, between the bioactive compounds, and on the second step, between their antioxidant capacities (DPPH, ABTS). Hierarchical Cluster Analysis (HCA) classified extracts into three clusters on the basis of measured antioxidant activity characteristics. Therefore, the result of this work indicates that roasted *Opuntia fusicus Indica* seeds could be used as a significant human nutrient.

**Keywords:** Seeds; Box–Behnken design; roasting; antioxidant activity; polyphenols; DPPH; ABTS; *Opuntia fusicus indica.*

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1. **Introduction**

*Opuntia fusicus – Indica* is notorious under the name of prickly pear; it belongs to the family of Cactaceae. It adapts well to arid and semi-arid conditions. It is also well spread in the Mediterranean region, Mexico, and South Africa [1–3]. Additionally, it is an important plant in the agricultural economies and has been cultivated since old periods [4,5]. Furthermore, prickly pears have been used for a long time in traditional medicine. Recent research showed that domestic use of seeds, fruits, and vegetables had a strong positive correlation in the prevention of modern diseases such as diabetes, cancer, and arthritis [6,7]. The seeds are
considered an essential source of nutritional values, such as flavonoids, polyphenols, fatty acids, and tannins, and their quantities are higher than in the fruit pulp[8]. In addition, they contain about 10 to 15 % of the edible pulp and are often discarded as waste after extraction from the pulp [9]. Roasting the seeds has numerous benefits such as promoting flavor, aroma, change the color and texture of roasted seeds, as well as eliminating unwanted microorganisms and food contaminants. Furthermore, they are known for deactivating enzymes that can accelerate the loss of nutrients and extending the duration of product life. In order to preserve the beneficial constituents of the seeds, the duration and temperature of roasting are considered the most important conditions [10–12]. Moreover, a number of research investigations have shown that the phenolic profile of different edible ingredients can be made of more health benefits by increasing their antioxidant capabilities [11]. This beneficial result is dependent on the grain considered and the roasting conditions utilized. During the roasting, the antioxidant compounds can degrade, and the formation of new products named Maillard reaction products (MRP) is observed. Degradation can influence antioxidant significantly [13]. Several studies assessed the effects of roasting on bioactive composition and antioxidant capabilities from various vegetable plants and seeds[14,15]. And it was found that the optimization of roasting conditions for seeds from Opuntia ficus Indica can significantly improve the antioxidant activity. Therefore systematic and precise optimization protocols for extraction and roasting conditions are required to maintain the bioactive and antioxidant activities precisely. Response Surface Methodology (RSM) is a statistical technique used to optimize the suggested protocols as it uses quantitative variables (roasting parameters) with an adequate experimental design to evaluate the effects of these parameters and their inherent interaction. Box-Behnken design (BBD) is an RSM capable of the generation of a mathematical model, taking into account the effect of the potential interactions among the factors under testing while keeping the number of experiments to be carried out minimized [14,16]. Therefore, the objective of the present work is to apply the RMS methodology and to investigate the effect of several factors such as the temperature of the roasting process, the duration of roasting, and the duration of extraction in order to optimize TCP (Total Phenolic Content), TFC (Total Flavonoids Content), TTC (Total Condensed Tannins Content), TSC (Total Sugars Content), and the antioxidant activity by DPPH and ABTS assays of Opuntia ficus Indica seeds. The correlation between the bioactive molecules and their antioxidant capabilities is evaluated by the Principal Component Analysis (PCA). A Hierarchical Cluster Analysis (HCA) was used to construct three clusters based on their bioactive compounds and antioxidant capacities.

2. Materials and Methods

2.1. Vegetals materials sites.

The plant seeds from Opuntia ficus Indica were harvested in the summer in the period between July and September 2019 in the Taza city region located in the East-North of Morocco.

2.2. Preparation of experimental extracts and extraction of antioxidant compounds.

After the material vegetal is collected (seeds), the seeds were isolated using water and dried in the dark at room temperature for 72 hours. Afterward, they are placed in an aluminum paper and roasted in a forced hot-air convection oven at different temperatures (60°C, 130°C, 200°C) for various durations (10,30,50min). The seeds were then crushed using a grinder. The resulting fine powder is stored at room temperature in an airtight container. Next, 50 g of fine
powder was extracted with ethanol solvent distilling between 40°C and 60 °C in a soxhlet. The solvent was evaporated by a rotary evaporator. The extract was stored at a temperature of about 4 °C for subsequent analysis.

2.3. Chemicals and reagents.

The chemical reagents used in this work are classified as follows: 2,2'-azino-bis (3-ethyl benzthiazoline-6-sulphonic acid), 2, 2-diphenyl-1-pyridazil (DPPH .90%), Ascorbic acid, aluminum chloride (ALCl₃), 2-thiobarbituric acid (TBA), KH₂PO₄, K₂HPO₄, sulfuric acid, potassium persulphate, phenol, ethylene-diamine, tetraacetic acid, Folin-Ciocalteau’s phenol reagent, sodium carbonate, sodium acetate, aluminum chloride, sodium hydroxide, ethanol, methanol, ferrous sulfate, ferric chloride, and 2,4,6-Tris (1-pyridyl )-5-traizine (TPTZ).

2.4. Total phenolic content (TPC).

The polyphenol content was done by the assay of Singleton et al. 1999[17]. 200 µl gent of seeds extracts was added to 1.5 ml of Folin-Ciocalteu reagent (10% diluted). After about 4 minutes of dilution, 1.5 ml of 5% sodium carbonate (Na₂CO₃) was added to the mixture and left in darkness. The results were collected after 2 hours by a spectrophotometer at 750 nm. The concentration of TPC was obtained according to a calibration interval using Galic Acid (0-100 µg/ml). The concentration of total polyphenols is expressed milligrams equivalent of a Gallic Acid gram of extract (mg EGA / g extract).

2.5. Total flavonoids content (TFC).

The aluminum trichloride (AlCl₃) assay described by Yeddes et al., 2013[18], is used to quantify the flavonoids in our extracts. One mL of each sample diluted is mixed with one ml of a solution of Aluminum chloride (AlCl₃) diluted to 2% in methanol. Consequently, the absorbance was measured at 430 nm after 10 minutes of the start of the reaction. Quercetin was used to establish a calibration curve. Therefore, the TFC was expressed in milligrams equivalent of quercetin per gram of extract (mg EQ / g extract).

2.6. Total condensed tannins content (TTC).

The analysis of TTC was obtained by Sun et al., 1998 [19]. 50 ml of diluted extract (50-600 µg/mL), 3 mL of 4% vanillin solution in methanol, and 1.5 of concentrated H₂SO₄ were added. The mixture was allowed to stand for 15 min, and the absorbance was measured at 430 nm, using catechin to establish a calibration curve. The TTC was expressed in mg catechin equivalent (CE) per gram of extract.

2.7. Total sugars content (TSC).

The TSC of Opuntia ficus Indica seeds extract was determined by Chaiwut et al. [20]. We used the phenol-sulfuric acid method. One mL of each extract was added to 1mL of aqueous phenol 5%. Then 5 mL of sulphuric acid was added to the mixture. After that, the mixture was incubated at 50°C for 20 min. The absorbance was read at 490 nm. The TTC was determined by a linear regression equation established using glucose. Therefore, the TSC was expressed in milligrams equivalent of glucose per gram of extract (mg D-GluE/ g extract).
2.8. Radical scavenging activity of DPPH (1-diphenyl picrylhydrazyl).

The free radical removal activity of the extracts was carried out using the method of Zengin et al. 2015 [21] with slight modification. A stable free radical, DPPH, was used. 1mL of each sample at different concentrations (50 to 1000 µg/mL) was added to 1 mL of DPPH methanol solution at 0.1 mM and left to stand for 30 min at 27°C. Both DPPH and methanol were used as controls. The mixture was incubated at 37°C in the dark for 20 min; after that, the absorbance was collected at 517 nm. The antiradical activity was estimated according to the following equation: % Radical scavenging activity DPPH = 1 - [A sample / A control] x 100, where A sample and A control are the absorbances of the sample and the control.

2.9. Radical cation inhibition activity (ABTS).

The radical cation activity of Opuntia ficus Indica extracts was estimated according to the method of Yim et al., 2013 [22]. 88 µL of 140 mM of potassium persulfate (K₂S₂O₈) was mixed with 5 mL of 7 mM ABTS⁺ solution. The solution was stored in the dark for 16 h at room temperature. Then, the absorbance of the solution was adjusted using ethanol to 0.70±0.05 at 734 nm. 10µL of Opuntia ficus Indica extract at different concentrations was added to one mL of ABTS⁺ solution (100 to 1000µg/mL). The absorbance was measured against the blank reagent at 734 nm. The inhibition activity was determined by the following equation: % Radical inhibition activity ABTS = 1 - [A sample / A control] x 100 where A sample and A control were the absorbances of the sample and the control.

2.10. Preparation of extracts according to the experimental design.

In this work, we used Box-Behnken Design (BBD); this technique consists of 15 experimental assays (Table 2). It was used for the optimization of roasting conditions and extraction factors. Three factors (independent variables) were optimized, which are roasting temperature, roasting time, and extraction time. These factors had 3 levels (-1, 0, +1), which are lower, medium, and higher, respectively. Table 1 presents the domains of variation and the coded levels of the three variables. TPC, TTC, TFC, TSC, and the antioxidant capacity by ABTS and DPPH assays were selected as the responses of model design (Y) of this work, and they are mentioned in Table 2.

| Extraction Process | Independent Variables | Level |
|--------------------|-----------------------|-------|
| soxhlet process    | X₁roasting temperature(°C) | 60    |
|                    | X₂roasting time (min)   | 10    |
|                    | X₃ extraction time (h)  | 3     |

The regression coefficients (β) were generated by the adjustment of the experimental results to a second-order polynomial model. The second-order polynomial model for response surface analysis was presented as follows [23]:

\[ Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \]

(1)

Where Y is the predicted response, as well as Xᵢ and Xⱼ were the independent variables. Moreover, β₀, βᵢ, βᵢᵢ, and βᵢⱼ was the regression coefficient of the mode (constant, linear, quadratic, and interactive effect), respectively. Furthermore, the analysis of variance (ANOVA) was used to obtain these regression coefficients using JMP11(SW) software. P-
value of the model, a p-value of lack of fit, coefficient of determination ($R^2$), and coefficient of adjustment ($R^2_{adj}$) were used to estimate the adequacy of the polynomial equation of the response. The desirability function was used for multi-response prediction [24]. The significance of all terms of the models was considered statistically different when the p-value <0.05. The surface graphs (3D) were visualized by JMP 11 (SW) software [25].

2.11. Principal component analysis (PCA), hierarchical cluster analysis (HCA), and matrix correlation.

The Pearson correlations between TPC, TFC, TTC, TSC, DPPH, and ABTS were performed by PCA, which represented the 15 samples according to their response values graphically in order to enable visual correlation of the data depending on the nature of the roasting condition and extraction time. HCA was performed to pursue the interrelatedness between all extracts into clusters on the basis of measured bioactive compounds characteristics. Moreover, the similarities between the analyzed extracts are represented in the form of dendrograms generated by the XLSTAT 2014 software. The distances between extracts were calculated using square Euclidean distances. This technique uses an analysis of variance approach to evaluate the distances between clusters [26].

2.12. Data analysis.

Box-Behnken Design (BDD) was applied in order to optimize the roasting factors and extraction time for extracts from *Opuntia ficus Indica* seeds using JMP 11 (SW) software. Moreover, PCA, HCA, and Pearson correlations were performed using XLSTAT 2014 software[27,28]. The IBM SPSS Statistics 21 software was used to present the results in means ± standard error of the mean. The significance of the results used by the Tukey test at alpha =0.05.

3. Results and Discussion

Optimization of roasting conditions and extraction time was carried out in 15 randomized trials. Table 2shows the data collected for TFC, TPC, TTC, TSC, ABTS (IC$_{50}$), and DPPH (IC$_{50}$) of extracts seeds from *Opuntia ficus Indica* from Morocco.

3.1. Second-order polynomial model and interpretation of the response surface model of TPC and TFC.

The TPC and TFC of extract seeds from *Opuntia ficus Indica* varied from 31.14±0.90 to 103.76 ±1.82 mg GAE / g extract and 26.14±0.98 to mgQE/g extract, respectively. According to data from ANOVA for TPC and TFC (Table 3), the models were significant; their p-value is <0.0001 and<0.0015, respectively. The coefficient of determination($R^2$) was 0.996167 and 0.976653 for TPC and TFC, respectively; these values indicated that the models are adequate because $R^2$ was more than 0.75[29].

| Run | $X_1$ | $X_2$ | $X_3$ | TPC | TFC | TTC | TSC | DPPH IC$_{50}$ | ABTS IC$_{50}$ |
|-----|------|------|------|-----|-----|-----|-----|----------------|---------------|
| 1   | 200  | 10   | 4.5  | 6.43±0.90$^a$ | 83.28±0.80$^a$ | 6.05±0.95$^{ad}$ | 40.32±0.91$^{a}$ | 205.35±1.21$^a$ | 711.89±2.1$^a$ |
| 2   | 60   | 50   | 4.5  | 4.52±1.29$^b$ | 36.45±0.71$^c$ | 1.23±2.0$^{ab}$ | 39.56±0.79$^b$ | 321.43±1.29$^b$ | 950.64±1.19$^e$ |
| 3   | 60   | 30   | 3    | 4.82±1.12$^e$ | 30.14±0.86$^f$ | 1.61±0.29$^a$ | 29.02±0.98$^f$ | 430.67±1.19$^f$ | 1090.15±1.95$^g$ |
| 4   | 130  | 50   | 6    | 66.38±1.19$^d$ | 48.54±1.40$^f$ | 6.88±0.41$^{ad}$ | 23.37±0.82$^{ad}$ | 219.20±1.08$^{ab}$ | 620.36±1.31$^c$ |
| 5   | 130  | 30   | 4.5  | 56.46±0.80$^{ab}$ | 44.03±0.97$^{ab}$ | 3.25±0.20$^{bc}$ | 30.17±0.93$^{bc}$ | 230.64±0.83$^b$ | 684.23±1.11$^f$ |

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Additionally, the adjusted determination coefficient $R_{aj}^2$ was 0.989267 and 0.934629 for the TPC and TFC, respectively, this indicates that the models adequately presented the true combination between all independents variables used. Moreover, the lack of fit was not significant for TPC and TFC because their p-values were 0.0673 and 0.0536, respectively, which indicated that the model equations were adequate for predicting the response values. Equations (2) and (3) can predict the effect of independent variables on the content of TPC and TFC, respectively. They were represented by a reduced regression model.

TPC (mg GAE/g extract) = 56.686667 + 25.50625X_1 + 12.06875X_2 + 4.1225X_3 + 2.77X_1X_2 + 1.5525X_1X_3 + 2.3575X_2X_3 + 10.641667X_1X_3 - 4.088333X_2X_3 - 6.095833X_3 + 1.76X_1 + 1.76X_3 (2)

TFC (mg QE/g extract) = 44.746667 + 27.6395X_1 + 7.53925X_2 + 0.62125X_3 + 3.3285X_1X_2 - 9.8825X_1X_3 - 0.005X_2X_3 + 21.671167X_1X_3 - 2.221333X_2X_3 - 5.180333X_3 (3)

The data of ANOVA for TPC (Table 2) showed that the factors $X_1$, $X_2$, and $X_3$ had a significant positive linear effect on TPC since their p-values were <0.0001, <0.0001, and 0.0043, respectively. Also, the quadratic effect $X_1^2$ had a significant positive effect (p-value=0.0003). On the contrary, the quadratic effects of $X_2^2$, $X_2^2$, and $X_3^2$ had a significant negative effect on TPC (p-value <0.05). Furthermore, no significant interaction between the three factors tested was observed (Table 2).

### Table 3. Data on the ANOVA of the regression coefficient and the terms of the model.

| Source | Coef | Sum of square | Degree of freedom | Mean square | F-avalue | p-avalue |
|--------|------|---------------|-------------------|-------------|----------|----------|
| TPC    |      | 7236.731      | 9                 | 804.081     | 144.382  | <0.0001* |
| Constant | 56.686667 |           | 1                 | 5204.5503   | 934.5376 | <0.0001* |
| $X_1$  | 25.50625 | 5204.5503     | 1                 | 1165.2378   | 209.2320 | 0.001*   |
| $X_2$  | 12.06875 | 1165.2378     | 1                 | 1165.2378   | 209.2320 | 0.001*   |
| $X_3$  | 4.1225  | 135.9601      | 1                 | 135.9601    | 24.4132  | 0.0043*  |
| $X_1X_2$ | 2.77   | 30.6916       | 1                 | 30.6916     | 5.5110   | 0.0658   |
| $X_1X_3$ | 1.5525 | 9.6410        | 1                 | 9.6410      | 1.7312   | 0.2454   |
| $X_2X_3$ | 2.3575 | 22.2312       | 1                 | 22.2312     | 3.9919   | 0.1022   |
| $X_1X_2X_3$ | 10.641667 | 418.1356     | 1                 | 418.1356    | 75.0811  | 0.0003*  |
| $X_1^2$ | -4.088333 | 61.7150     | 1                 | 61.7150     | 11.8016  | 0.0208*  |
| $X_2^2$ | -6.095833 | 137.2031    | 1                 | 137.2031    | 24.6364  | 0.0042*  |
| Residual | 27.8456 | 5              |                   | 5.569       |          |          |
| Total   | 7264.5767 |                 | 14                | 88.8091     | 14.0330  | 0.0673   |
| Lack of fit | 26.582725 | 3              |                   | 8.86091     |          |          |
| Pure Error | 1.262867 | 2              |                   | 0.63143     |          |          |
| Total Error | 27.845592 | 5              |                   | 5.569       |          |          |
| $R^2$  | 0.9996167 |             |                   |             |          |          |
| $Radj^2$ | 0.989267 |             |                   |             |          |          |
Concerning the results of ANOVA for TFC, the roasting temperature (X₁) and roasting time (X₂) had a positive linear effect on TFC (p-value<0.05). However, the extraction time (X₃) was not significant (p-value>0.05). In addition, the interaction effect between X₁ and X₃ had a significant negative effect on TFC (p-value>0.05). Furthermore, the quadratic effect of roasting temperature (X₁) had a significant negative effect on TFC (p-value=0.0014).

3.2. Response surface methodology (RSM) analysis.

The effects of three factors: roasting temperature, roasting time, and extraction time, as well as their interaction and quadratic effect on TPC and TFC were visualized on response surface (3D) plots shown in Figure 1. Accordingly, Figure 1a shows TPC as a function of roasting temperature (X₁) and roasting time (X₂) at fixed extraction time (X₃) (6h). It showed that TPC rapidly increased when the roasting temperature (X₁) increased at a roasting time (X₂) fixed, as well as it increased gradually when the roasting time (X₂) increased and reached an optimum in the region close to 200°C and 50min. Figure 1b shows the effect of roasting time (X₂) and extraction time (X₃) at fixed roasting temperature (200°C) on the TPC; the TPC increased with increasing extraction time (X₃) when the roasting time (X₂) was fixed, as well as, it increased gradually when the extraction time increased before passing 5h of extraction, after that, it decreased. The effect of roasting temperature (X₁) and extraction time (X₃) at a fixed roasting time (X₂) (50°C) is shown in Figure 1c; accordingly, TFC increased with increasing roasting time when the extraction time was fixed. Also, when the roasting temperature was fixed, the TFC increased before the extraction time increased at 5h; after that, it decreased marginally. Moreover, the optimization of the response values was carried out according to desirability function (d) in order to achieve the optimal response for TPC, for instance. The optimum response for TPC was found when the desirability was close to 1 (Figure 4)[24,30,31]. Therefore, the optimum of TPC was at roasting temperature 200°C, roasting time 50 min, and extraction time 5.49 h with 106.2311mg GAE/g extract predicted response and desirability d=0.94(Figure. 4a). These results were consistent with the previous studies of TPC. Chandrasekara and Shahidi,2011[32] indicated that the roasting at high temperatures (130°C) for 33 min increased the TPC significantly more than the raw seeds (testa, cashew nuts). Also, the TPC (in both water and ethanol) from peanut skin increased by about 35% relative to the raw sample at a roasting temperature of 175 °C for 5 min[33]. Moreover, in the study done by
Locatelli *et al.* [34], the TPC of hazelnut skin (extract soluble) increased at 180°C for 20 min more than 10 min. In addition, Yin *et al.* [35] showed that the TPC increased about 2.4-3.4 times at a temperature between 120°C and 140°C for 180 min. Moreover, the TPC significantly increased (p-value <0.05) at a heat treatment [36].

Concerning the TFC, Figure 1d shows the response surface plot of roasting temperature ($X_1$) and roasting time ($X_2$) on the total flavonoids content when the extraction time ($X_3$) was fixed at 3 h. The TFC was stable when the roasting time was fixed, and the roasting temperature increased between 60-120 °C; beyond 120 °C, the TFC quickly increased. Also, the TFC slightly increased with increasing roasting time ($X_2$) when the roasting temperature was fixed. In addition, Figure 1e showed that the effect of roasting time ($X_2$) and extraction time ($X_3$) on the TFC when the roasting temperature was fixed at 200°C; the TFC increased with the increasing of the roasting time ($X_2$) when the extraction time fixed, but, it decreased with the increasing of the extraction time ($X_3$) when the roasting time ($X_2$) fixed.

Moreover, the effect of the roasting temperature ($X_1$) and extraction time ($X_3$) on the TFC when the roasting time ($X_2$) was fixed at 50°C (Figure1f), according to that, the TFC
slightly increased with the increasing of extraction time when the X1 fixed. Also, the TFC quickly increased with the increasing of roasting temperature when the X3 was fixed. The strong level of TFC was recorded at a higher roasting temperature. Consequently, the maximum of TFC was at roasting temperature 200 °C, roasting time 50 min, and extraction time 3.16h with 106.85 mgQE/g extract predicted response and the desirability is d=0.948(Figure 4b). These results are similar to various works. Lin et al. [37] indicated that the TFC increased significantly by roasting (after 5 min); in addition to that, the flavonoid aglycones and acids are increased by the effects of roasting temperature and roasting time. Furthermore, a study was done by Kumar et al.[48] showed that the fraction of sugar in flavonoids glycosides had an essential role in antioxidant activity, and the aglycones had a strong effect on the antioxidant activity more than the glycosides. The results of TPC and TFC of this study after the roasting can be explained by these causes. First, the phenolic molecules can be degraded/polymerized during the roasting, which showed the formation of new molecules; these molecules can be more soluble in water and ethanol. Also, these molecules can be reagent with the Folin-Ciocalteu in the alkaline middle [33]. Secondly, during the roasting, the bound compounds bioactive can be released [39].

3.3. Second-order polynomial model and interpretation of the response surface model of TTC and TSC.

In this work, the TTC and TSC of extract seeds from Opuntia ficus Indica varied from 1.23±0.20 to 9.12±0.90 mg QAE/g extract and from 10.35±0.32 to 44.62±0.79 mg D-Glu E/g extract, respectively. According to data of ANOVA presented in Table 4, the models were significant because their p-value was 0.0023 and 0.0004 for TTC and TSC, respectively.

| Source | Coef | Sum of square | Degree of freedom | Mean square | F-value | p-value |
|--------|------|---------------|------------------|-------------|---------|---------|
| TTC    |      |               |                  |             |         |         |
| Model  | 3.4433333 | 87.863198 | 9 | 9.76258 | 19.1480 | 0.0023* |
| Constant  | | | | | | |
| X1  | 2.80625 | 63.000313 | 1 | 63.000313 | 123.5668 | 0.0001* |
| X2  | 1.09875 | 9.658013 | 1 | 9.658013 | 18.9429 | 0.0073* |
| X3  | 0.795 | 5.056200 | 1 | 5.056200 | 9.9171 | 0.0254* |
| X1*X1 | 0.845 | 2.856100 | 1 | 2.856100 | 5.6019 | 0.0642 |
| X1*X2 | 0.6625 | 1.755625 | 1 | 1.755625 | 3.4344 | 0.1227 |
| X2*X2 | 0.8275 | 2.739025 | 1 | 2.739025 | 5.3722 | 0.0862 |
| X1*X3 | 0.7983333 | 2.352421 | 1 | 2.352421 | 4.6156 | 0.0844 |
| X2*X3 | 0.2433333 | 0.218626 | 1 | 0.218626 | 0.4288 | 0.5415 |
| X3*X3 | -0.219167 | 0.177356 | 1 | 0.177356 | 0.3479 | 0.5810 |
| Residual | | 2.549242 | 5 | 0.50985 | | |
| Total | | 90.412440 | 14 | | | |
| Lack of fit | | 2.4593750 | 3 | 0.819792 | 18.2446 | 0.0524 |
| Pure Error | | 0.0898667 | 2 | 0.044933 | | |
| Total Error | | 2.5492417 | 5 | | | |
| R2 | 0.971804 | | | | | |
| R Adj2 | 0.921052 | | | | | |
| TSC    |      |               |                  |             |         |         |
| Model  | 30.296667 | 1107.8079 | 9 | 123.090 | 38.2405 | 0.0004* |
| Constant  | | | | | | |
| X1  | -3.7025 | 109.66805 | 1 | 109.66805 | 34.0707 | 0.0021* |
| X2  | -5.41 | 234.14480 | 1 | 234.14480 | 72.7421 | 0.0004* |
| X3  | -5.71 | 260.83280 | 1 | 260.83280 | 81.0333 | 0.0003* |
| X1*X1 | -7.3575 | 216.53123 | 1 | 216.53123 | 67.2701 | 0.0004* |
| X1*X2 | -5.2525 | 110.35503 | 1 | 110.35503 | 34.2842 | 0.0021* |
| X2*X2 | 3.5075 | 49.21022 | 1 | 49.21022 | 15.2882 | 0.0113* |
| X1*X3 | -1.652083 | 9.83519 | 1 | 9.83519 | 3.0555 | 0.1409 |
| X2*X3 | 3.9179167 | 56.67719 | 1 | 56.67719 | 17.6080 | 0.0885* |
Moreover, the lack of fit was not significant (p-value >0.05), which indicated that the models' equation was for p adequate for predicting the response values. In addition to that, the R² values were 0.971804 and 0.98568 for TTC and TSC, respectively, which indicates that 97% of experimental results were compatibles [40]. These values showed that the models adequately presented the true combinations between all variables used. Furthermore, the adjusted determination coefficients R_adj² were 0.921052 and 0.959904 for TTC and TSC, respectively, which indicated that the models were adequate (R_adj²>0.75)[29]. Consequently, the second-order polynomial models were applied in equations 4 and 5 for TTC and TSC, respectively. 

\[
\text{TTC(mgQAE/gextract) =3.4433333+2.80625X_1+1.09875X_2+0.795X_3+0.845X_1^2+0.6625X_1^3+0.8275X_2^2+0.7983333X_1X_2+0.2433333X_2X_3+0.219167X_3^2 \cdot X_3} \\
\text{TSC(mgD-GluE/gextract) =30.296667-3.7025X_1-5.41X_2-5.71X_3-7.3575X_1X_2-5.2525X_1^3+3.5075X_2+X_3-1.632083X_1X_3+3.9179167X_2X_3-3.727083X_3^2 \cdot X_3} 
\]

According to Table 4, the linear effect of roasting temperature (X_1), roasting time (X_2), and extraction time (X_3) had a significant positive effect on TTC (p-value <0.05). Moreover, the interaction effects between X_1,X_2, and X_3 were not significant on TTC (p-value> 0.05). Also, the quadratic effect of three independent variables had no significance on TTC because their p-value was 0.0844, 0.5415, and 0.5810 for X_1, X_2, and X_3, respectively. As regards TSC, the linear effect of roasting temperature, roasting time, and extraction time had a significant negative effect (p-value < 0.05). Additionally that, the interaction effects X_1 * X_2 and X_2 * X_3 had a significant negative effect on TSC (p-value > 0.05); on the contrary, the interaction effect between roasting time and extraction time had a significant positive effect on TSC because of its p-value is equal 0.0113. Furthermore, the quadratic effects X_2*X_2 had a significant positive effect on TSC (p-value =0.0085), but the quadratic effect of extraction time X_3*X_3 had a negative effect on TSC (p-value=0.0104). On the contrary, the quadratic effect of X_2*X_2 was not significant (p-value>0.05).

### 3.4. Response surface methodology (RSM) analysis.

The responses surfaces (3D) of regression equations 4 and 5 were established by RSM to illustrate the effects of each independent variable studied on TTC and TSC (Figure 2). According to that, Figure 2a shows the influence of roasting temperature (X_1) and roasting time (X_2) on TTC when the extraction time (X_3) was fixed at 6h. It noted that the TTC increased with the increasing of the roasting temperature when the roasting time was fixed. Also, it is shown that the roasting time (X_2) had an influence on the TTC because the TTC slowly increased with the increase of X_2 when the X_1 was fixed. Moreover, the positive interaction effectX_1 * X_2 on the TTC was observed. Figure 2b shows the effect of roasting time and extraction time on the TTC when the X_1 was fixed at 200°C. Thus, the positive interaction X_2
* X₃ was recorded. Therefore, the TTC increased with the increase of the X₂ and X₁, respectively. Figure 2c showed the effect of roasting time and extraction time on the TTC when the roasting temperature was fixed at 50 min. The positive effect of X₂ and X₃ on the TTC was observed. Therefore, the TTC increased with the increase of two factors X₁ and X₂. It reached a maximum of TTC in the region close to 50 min and 6 h. The maximum of TTC was at roasting temperature: 200 °C, roasting time 50 min, extraction time 6 h with 11.30 mg QAE/g extract predicted responses, and the desirability is d = 0.99 (Figure 4c). These results are agreed with those reported by Lin et al. [37]. They indicated that the ethanolic extracts had a strong level in the TTC during the roasting (200 °C for 20 min).

Concerning the data of TSC, figure 2d shows the effect of roasting temperature and roasting time on TSC when the extraction time fixed (6 h); accordingly, the negative interaction effect between X₁ and X₂ was recorded; thus, the optimum of TSC was observed in the region at high temperature 200 °C and less roasting time 10 min. Moreover, Figure 2e presents the effect of roasting time and extraction time on the TSC when the roasting temperature was fixed at 200 °C; the TSC decreased with the increasing of the extraction time when the roasting time fixed, the same effect when the X₃ fixed.

**Figure 2.** Response surface plots of roasting conditions of TTC (a, b, and c) and TSC (d, e, and f) of the *Opuntia ficus Indica* seeds extracts.
According to Figure 2f, who showed the influence of X1 and X3 on the TCS when the X2 was fixed at 10min, the TSC quickly decreased with the increase of X3 when the X1 fixed, but it slowly decreased when the increasing of X1 and X3 fixed. These results are similar to work showed that the content of free sugar generally decreased with increasing roasting temperature and time[41]. Because during the various roasting, nutrients such as carbonyl and amino molecules can be degraded by non-enzymatic browning reactions [42,43]. Therefore, the optimum of TSC was at roasting temperature: 60 °C, roasting time 50 min, extraction time 5.12 h with 38.86 mg D-Glu E /g extract predicted responses, and the desirability is d =0.81(Figure 4d).

3.5. Second-order polynomial model and interpretation of the response surface model of DPPH and ABTS assays.

ANOVA data from DPPH and ABTS assays based on the RMS are mentioned in Table 5. The p-value of the models was < 0.0001, which showed that the models were significant. In addition to that, the lack of fit was not significant because it was 0.0756 and 0.0504 for DPPH and ABTS assays, respectively, which confirms the adequacy of the models to predict the antioxidant activity from extracts seeds roasted. Moreover, the R2 and R2 adj were 0.999899 and 0.999718, respectively, for DPPH, and they were 0.999813 and 0.999477, respectively, for ABTS. Because of R2> 0.75, which indicates the adequacy of the models[29]. Consequently, the second-order polynomial models were applied in equations 6 and 7.

DPPH (IC50)(µg/mL) =231.41-132.5163X1-74.9875X2-24.31375X3-10.35X1·X2+2.9525X1·X3+5.34X2·X3+7.28875X1·X3+35.04125X2·X3+39.67275X3·X3  

ABTS(IC50)(µg/mL) =682.44333-264.7125X1-150.9038X2-62.55125X3+45.4125X1·X2-21.3625X1·X3+5.76X2·X3+30.453333X1·X3+72.955833X2·X3+76.280833X3·X3  

According to the statistical analysis of ANOVA for DPPH assay, roasting temperature (X1), roasting time (X2), and extraction time (X3) had a negative significance (p-value<0.05) linear effect on the IC50 of DPPH assays. Also, their quadratic effect X1 * X1, X2 * X2, and X3 * X3 had a positive significant(p-value<0.05) effect on the IC50 of DPPH assay.

| Source       | Coef         | Sim of square | Degree of freedom | Mean square | F-value | p-value |
|--------------|--------------|---------------|-------------------|-------------|---------|---------|
| DPpH(C50)    |              |               |                   |             |         |         |
| Model        | 231.41       |               | 9                 | 22267.0     | 5520.906| <0.0001*|
| Constant     | -132.5163    | 140484.45     | 1                 | 140484.45   | 34831.88| <0.0001*|
| X1           | -74.9875     | 44985.00      | 1                 | 44985.00    | 11153.63| <0.0001*|
| X2           | -24.31375    | 4729.27       | 1                 | 4729.27     | 1172.580| <0.0001*|
| X3           | -10.35       | 428.49        | 1                 | 428.49      | 106.2403| <0.0001*|
| X1 * X2      | -2.9525      | 34.87         | 1                 | 34.87       | 8.6455  | 0.0322* |
| X1 * X3      | 5.34         | 114.06        | 1                 | 114.06      | 28.2808 | 0.0031* |
| X2 * X3      | 7.28875      | 196.16        | 1                 | 196.16      | 48.6354 | 0.0009* |
| X1 * X1      | 35.04125     | 4533.74       | 1                 | 4533.74     | 112.4102| <0.0001*|
| X2 * X2      | 39.67275     | 5811.72       | 1                 | 5811.72     | 1440.964| <0.0001*|
| Residual     | 20.17        | 5             | 4.0               |             |         |         |
| Total        | 200423.16    | 14            |                   |             |         |         |
| Lack of fit  | 19.136275    | 3             | 6.37876           | 12.3884     | 0.0756  |         |
| Pure Error   | 1.029800     | 2             | 0.51490           |             |         |         |
| Total Error  | 20.166075    | 5             |                   |             |         |         |
| R2           | 0.999899     |               |                   |             |         |         |
| Radj2        | 0.999718     |               |                   |             |         |         |
Moreover, the interaction effect X2 * X3 had a significant positive effect on DPPH (IC50) because its p-value is equal 0.0031, on the contrary, the interaction effects X1 * X2 and X1 * X3 had a negative significant (p-value<0.05) effects on IC50 DPPH. Concerning data of ANOVA for ABTS assay. The three factors X1, X2, and X3 had a negative linear effect on IC50 ABTS (p-value<0.05); in addition to that, their quadratic effects had a positive significant effect on IC50 ABST(p-value<0.05). In addition to that, the interaction effect between roasting temperature (X1) and extraction time (X3) had a significant negative effect on IC50 ABTS (p-value<0.0001). On the contrary, the interaction effect X2 * X3 had not significant because its p-value is 0.0924.

3.6. Response surface methodology (RSM) analysis.

The response surface (3D) of regression equations 6 and 7 were constructed using RMS and are shown in Figure3. The antioxidant activity is inversely proportional to IC50. Moreover, Figure 3a and Figure 3d show the effect of roasting time(X2) and roasting temperature (X1) on the IC50 for DPPH and ABTS assays, respectively, when the extraction time (X3) was fixed at 4.5h. We observed that the antioxidant activity by DPPH and ABTS assays increased significantly with the increasing of roasting time (X2) and roasting temperature (X1) because of the IC50 of DPPH and ABTS assays was decreased. Therefore, the maximal of the antioxidant activity for the two assays were in the at a high roasting temperature region (X1) and roasting time (X2). Additionally, the effects of roasting time(X2) and extraction time (X3) on IC50 when the roasting temperature was fixed(200°C) are presented in Figure 3b and Figure 3e for DPPH and ABTS assays, respectively. The negative effects significant of X2 and X3 on IC50 for DPPH and ABTS are recorded. Thus, the antioxidant activity increased with the increasing extraction time and roasting temperature for the two assays. Figure 3c and Figure 3f showed the effects of roasting temperature and extraction time when the roasting time was fixed(50min) on IC50 of DPPH and ABTS, respectively. We observed that the antioxidant activity increased for the two assays with the increasing of two factors X1 and X3 because the IC50 value was decreased. Moreover, the optimal antioxidant activity was at a strong roasting temperature and extraction time for DPPH and ABTS assays. Based on the desirability function, the maximum of antioxidant activity by DPPH assay was at a roasting temperature...
(X₁=200 °C), roasting time (X₂=47.85 min), and extraction time (X₃=4.91) with 98.226% of inhibition, which matches 73.21 µg/mL predicted response, as well as, the desirability is d=0.84 (Figure 4e).

![Response surface plots of roasting conditions of DPPH IC₅₀ (a, b, and c) and ABTS IC₅₀ (d,e, and f) of the *Opuntia ficus Indica* seeds extracts.](image)

**Figure 3.** Response surface plots of roasting conditions of DPPH IC₅₀ (a, b, and c) and ABTS IC₅₀ (d,e, and f) of the *Opuntia ficus Indica* seeds extracts.

For ABTS assay, the optimal for the antioxidant activity was at a roasting temperature (X₁=200°C), roasting time (X₂=49.min), and extraction time (X₃=5.28) with 97.38% of inhibition, which matches 305.57 µg/mL predicted response, as well as, the desirability is d=0.907 (Figure 4f.).

These results confirm those found by several works. Lin *et al.* [37] showed that the strongest antioxidant activity was recorded at high roasting temperature for ethanolic almond extract (*Prunus dulcis*) kernel, as well as the power for scavenging DPPH radical during the roasting temperature at 200 °C for 20 min was more than the raw sample. In addition, Chandrasekara and Shahid [32] showed that the antioxidant activity for DPPH radical increased significantly with increasing the roasting temperature for soluble phenolic *testa* extract. Concerning ABTS assays, Yin *et al.* [35] indicated that the scavenging (ABTS) increased during the roasting at 130 °C-140 °C after 60 min. In addition, Gao *et al.* [44] reported that the activity (ABTS) increased significantly at 160 °C for 10 min of roasting compared to raw extract.
Table 6. Experimental and predicted results at conditions optimal.

| Responses                  | X1 roasting temperature (°C) | X2 roasting time (min) | X3 extraction time(h) | Predicted value | Experimental value |
|----------------------------|-----------------------------|------------------------|-----------------------|-----------------|--------------------|
| Total phenolic Contents    | 200°C                       | 50min                  | 5.49 h                | 106.23          | 103.5±0.76         |
| (mgGAE/gextract)           |                             |                        |                       |                 |                    |
| Total flavonoids           | 200°C                       | 50min                  | 3.16h                 | 106.85          | 104.29±0.54        |
| content(mgQE/gextract)     |                             |                        |                       |                 |                    |
| Total Tannins Content      | 200°C                       | 50min                  | 6h                    | 11.30           | 10.16±0.89         |
| (mgQAE/gextract)           |                             |                        |                       |                 |                    |
| Total sugars Content       | 60°C                        | 50min                  | 5.12h                 | 38.86           | 40.35±0.99         |
| (mg D-Glu Egextract )      |                             |                        |                       |                 |                    |
| DPPH IC50 (µg/mL)          | 200°C                       | 47.85min               | 4.91h                 | 73.21           | 75.48±0.85         |
| ABTSIC50 (µg/mL)           | 200°C                       | 49.69min               | 5.28h                 | 305.57          | 306.59±0.90        |

These results can be justified as follows: during roasting, the antioxidants characteristics can be enhanced due to a reaction between amino acids and the reducing sugars; this reaction produced a new molecule named Maillard reaction products (MRPs), as well as the degradation of the heat-labile antioxidants compounds [32,45]. Moreover, during the thermal treatment, the bound antioxidant phenol and bound polymeric compounds of plants may be degraded and released, which leads to an increase in the antioxidant capacity[46]. Furthermore, the resultant melanoidin and Maillard reaction products had a higher antioxidant activity, which depends on the presence of reductone-type structures[47], as well as, roasting improves the solubility of non-phenolic molecules[48].

3.7. Verification and comparisons of predicted models and experimental values.

The validity of the predictive models was checked by comparing the results predicted and experimental data. This verification was carried out using the experimental conditions of the maximal response values. Based on data from Table 6, the predicted results are close to experimental results.
3.8. Correlation matrix.

The correlation coefficients and the p-values of all responses studied were presented in Table 7 and Table 8, respectively. In addition, the antioxidant activities (ABTS (1/IC$_{50}$); DPPH (1/IC$_{50}$)) expressed the power to inhibit ABTS and DPPH radical, respectively; they are inversely proportional to IC$_{50}$. 

Figure 4. Desirability results for a: TPC, b: TFC, c: TTC, d: TSC, e: DPPH, and f: ABTS.
Table 7. Pearson’s correlation matrix between antioxidant activities and bioactive compounds.

| Variable | TPC   | TFC    | TTC   | TSC   | DPPH (1/IC_{50}) | ABTS (1/IC_{50}) |
|----------|-------|--------|-------|-------|------------------|------------------|
| TPC      | 1     |        |       |       |                  |                  |
| TFC      | 0.917 | 1      |       |       |                  |                  |
| TTC      | 0.949 | 0.831  | 1     |       |                  |                  |
| TSC      | -0.560 | -0.358 | -0.607 | 1     |                  |                  |
| DPPH (1/IC_{50}) | 0.917 | 0.836  | 0.891 | -0.650 |                  |                  |
| ABTS (1/IC_{50}) | 0.920 | 0.802  | 0.907 | -0.695 | 0.992            | 1                |

The results in bold are different from 0 at a significance level alpha=0.05.

Table 8. p-value of coefficient correlations matrix.

| Variables | TPC     | TFC     | TTC     | TSC     | DPPH(1/IC_{50}) | ABTS(1/IC_{50}) |
|-----------|---------|---------|---------|---------|----------------|----------------|
| TPC       | 0       |         |         |         |                |                |
| TFC       | < 0.0001 | 0       |         |         |                |                |
| TTC       | < 0.0001 | 0.000  | 0       |         |                |                |
| TSC       | 0.030   | 0.191   | 0.016   | 0       |                |                |
| DPPH(1/IC_{50}) | < 0.0001 | 0.000  | < 0.0001 | 0.009 | 0              |                |
| ABTS(1/IC_{50}) | < 0.0001 | 0.000  | < 0.0001 | 0.004 | < 0.0001       | 0              |

The results in bold are different from 0 at a significance level alpha=0.05.

As can be seen from Tables 7 and 8, the strong positive correlations (p-value < 0.0001) between the phytochemical compounds (TPC, TFC, TTC) and the antioxidant power were recorded. The coefficient correlations of TPC were 0.917 and 0.920 with free radical scavenging DPPH and ABTS, respectively. These positive correlations mean that the antioxidant activity of *Opuntia ficus Indica* seeds extracts can be attributed to the presence of polyphenol (TPC). They were also reported by various researchers [49,50]. Strong significant positive correlation between TFC and antioxidant power between DPPH (1/IC_{50})(r^2 = 0.836), and ABTS(1/IC_{50}) (r^2 = 0.802) was observed. Moreover, it was found that the TTC also had a significant positive correlation (p-value < 0.0001) between DPPH(1/IC_{50}) and ABTS(1/IC_{50}). Accordingly, these correlations are confirmed by high positive significant correlations (p-value < 0.05) between TPC, TFC, and TTC. We also observed that the TSC was not significant with antioxidant activity (p-value > 0.05). In addition, the higher positive correlation was significant between 2 antioxidant activity (r^2 = 0.992) and showed that the same antioxidant compounds in our samples are responsible for the scavenging activity of 2 free radicals ABTS and DPPH.

3.9. Principal component analysis PCA.

Figure 6 showed the projections of the results of experimental assays (15 extracts) by the factorial plan (F1-F2), according to PCA. The first essential component, F1, explains 83.38% of the information, and the second shows 11.64% of the information. Thus, the cumulative percentage was 95.02%; it is more than 50%, which indicated that the linear combination is already representative of the variables. The correlations between all responses are shown in Figure 5 by the plane formed by axes F1 and F2. Therefore, the F1 plan was constructed by the positive correlation between TFC, TPC, TTC, ABTS (1/IC_{50}), and DPPH (1/IC_{50}). But the F2 plan was formed by TSC (Figure 5). The extracts of this study were separated into 3 groups based on responses results in Figure 6.
Figure 5. Principal Component Analysis factorial plan carried out on the values (TFC, TPC, TSC, TTC, ABTS (1/IC50), and DPPH(1/IC50)).

Group I is composed of four extracts (1, 13, 7, 11); these extracts had high values of TPC, TFC, and TCC; also, they had a high antioxidant capacity by ABTS and DPPH assays. On the contrary, they had a lower value of TSC. Group II contains 6 extracts (8, 3, 6, 2, 12, and 14). The extracts of this group are characterized by the low values of TPC, TFC, and TTC, but they had a higher value of TSC. Its antioxidant capacity is low compared to other groups. Group III is formed by five extracts (5, 10, 9, 4, 15). The extracts of this group are characterized by a higher value of TSC and by medium values of TPC, TFC, and TTC. Consequently, their antioxidant capacity is medium compared to G I. Group I contains extracts characterized by a strong roasting temperature of 200 °C, which indicated that their antioxidant activity is stronger and more than the extract of G II and G III. Therefore, roasting can increase the extraction of bioactive molecules responsible for antioxidants activity.
3.10. Hierarchical clustering analysis HCA.

According to HCA, extracts were classified by the squared Euclidean and Wards method to estimate the similarity measure. HCA was used to evaluate the correlation between the extracts and to present similarities of 15 extracts (experiment assays) based on data of antioxidant contents and antioxidant activity, as indicated in the dendrogram in Figure 7. According to bioactive molecules, the 15 extracts were clustered into three clusters. Cluster I contains 4 extracts (1, 13, 7, 11) representing for 26.66% of the total extracts characterized by a high range mean of TPC, TFC, and TTC 87.74 mg GAE/g extract, 90.36 mg QE/g extract, and 7.06 mg QAE/g extract, respectively, as well as, they had a stronger antioxidant capacity. Cluster II, the largest cluster formed using 6 extracts, namely 8, 3, 6, 2, 12, and 14, accounted for 40% of total extracts; these extracts had the lowest mean value of TPC, TFC, and TTC 35.57 mg GAE/g extract, 33.60 mg QE/g extract, and 1.62 mg QAE/g extract respectively. Also, they had a high mean level of TSC 33.73 mg D-Glu E/g extract, and they are characterized by a low antioxidant activity by DPPH and ABTS. From the cluster analysis, Cluster III contained four extracts, namely 5, 10, 9, 4, and 15 representing for 33.33% of the total extracts, with a medium mean values of antioxidant compounds TPC, TFC, TTC, and TSC 57.91 mg GAE/g extract, 44.46 mg QE/g extract, 4.06 mg QAE/g extract, and 28.06 mg D-Glu E/g extract respectively, as well as, a medium antioxidant power. These results are in agreement with the data of the PCA, in which the distribution of all extracts on the score plot indicates a similar trend. Furthermore, the PCA results were consistent with those of HCA.

4. Conclusions

RSM (response surface method) was employed to optimize the conditions of the dry thermal processing in order to determine the optimum levels of bioactive molecules and their antioxidant activities from Opuntia ficus Indica seeds extracts. The results showed that the levels of bioactive molecules increased significantly with increasing roasting temperature and roasting time. Moreover, the experimental values generated based on the optimized roasting factors were found close to the predicted values. Therefore, this work suggests that the models obtained can be used to optimize the roasting temperature, roasting time, and extraction time for the extraction of bioactive molecules from Opuntia ficus Indica seeds. PCA indicated a positive correlation between bioactive molecules and antioxidant activities (ABTS, DPPH). Furthermore, HCA showed that the interrelatedness between extracts. Therefore, roasted
**Opuntia ficus Indica** seeds may be considered as traditional food with strong antioxidant power for the production of functional foods.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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