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Research Article

Optimization of Drying Conditions of *Eruca Vesicaria* Leaves and Study of Their Effects on Phenolic Compounds and Antioxidant Activity Using Response Surface Methodology

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Abstract

In this study, the optimization of *Eruca verisacira* leaves drying parameters and study of their effects on total phenols content (TPC), total flavonoids content (TFC) and antioxydant activity tested by DPPH essay and ABTS essay were realized. An experimental design was formulated using the Design-Expert software (version 9.0.3.1) with 2^3 factorial designs. eleven experiments were realized, and data and results were analyzed using the Response Surface Methodology (RSM). The analysis of variance shows a significant linear effect of drying time and heat treatment on total phenols content and total flavonoids content. It's shows that the increase in the duration and temperature of the heat treatment can allow a better quantification of the phenolic compounds. Results indicate a significant negative linear effect of temperature on the antioxidant activity of the extracts. This shows that antioxidant activity increases with temperature. Results showed a negative and significant linear effect of Time/Temperature interaction for the antiradical activity on DPPH and $ABTS^{+}$. Knowing that the effect of time is not significant, the effect of the interaction of the two parameters follows the trend of the results of the effect of temperature. The optimal heat treatment conditions (100°C for 30 min), selected by the Design-Expert statistical software, present the best desirability value of around 0.77. The conformity of the experimental values with those predicted by the mathematical models confirms the choice of the optimum 100°C for 30 min.

Keywords: Heat Treatment Conditions; Optimization; Antioxidant Activity; Phenolic Compounds

Abbreviations

TPC: Total Phenols Content; TFC: Total Flavonoids Content; DPPH: 2,2-Diphényl-1-Picrylhydrazyl; ABTS: Acide 2,2' Azino-Bis 3-Éthylbenzothiazoline-6-Sulfonique; RSM: Response Surface Methodology; EC50: Concentration Required to Scavenge 50% DPPH Free Radicals

Introduction

Arugula salad (*Eruca vesicaria*) is a plant belonging to the Brassicaceae family (or Cruciferae) [1,2]. In recent years, these crops have gained greater importance as vegetables and culinary herbs, especially among Middle Eastern and European populations [3]. It is widely consumed fresh by human, as salad, or prepared as a

steamed vegetable or used as a spice or food ingredient (Kim and Ishii, 2006). In the last two decades, rocket has become very popular and widely produced by fresh-cut industries because of its short biological cycle (40- 60 days) and its spicy hot taste [4,5]. Epidemiological studies have indicated that consumption of Brassica vegetables is associated with many health-promoting effects, which are generally attributed to bioactive compounds such as glucosinolate (GSL), derived degradation products and phenolic compounds [6,7]. Phenols, which are important because of their antioxidant activity and free radical-scavenging activities and generally positive effects on human health [7]. Previous studies showed that high drying temperatures promote the degradation of some bioactive compounds such as phenolic acids, flavonoids, and anthocyanins in different varieties of food [8]. In recent studies, Response Surface Methodology (RSM) acts as an important implement for process optimization [9]. It uses quantitative data from an appropriate experimental protocol to simultaneously determine and solve multidimensional problems [10]. The equations describe the effect of test variables on responses, determine interactions between test variables, and represent the combined effect of all test variables in any response [10]. It is an appropriate method to design, improve, and formulate the process parameters for the development of novel goods and similarly to modify the current produces properties for better retention of its physicochemical, nutritional, and chemical composition. RSM substantially chosen as a proficient technique to enhance such practices [9]. While it is known that thermal processes manipulate the physicochemical and biological characteristics of phytochemicals [11], so far, the impacts of heating on the quality of arugula salad (*E.vesicaria longirostris)* for an eventual spice use have not been reported. Hence, phenolic compounds and antioxidant activity in arugula leaves with the impact of heat processing on their quality were investigated. The main objective of this study, is to determine an optimum pair of drying parameters (time and temperature) leading to better quantification of phenolic compounds and better antioxidant activity in *E.vesicaria* leaves.

Materials and Methods

Reagents and solutions

Folin-Ciocalteau reagent was provided by (PanReac, EU) and solution was prepared daily in water. Methanol, Ethanol, Sodium bicarbonate and Gallic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Sodium bicarbonate and ABTS were dissolved in water.

ABTS (diammonium 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonate)). Quercetin was provided by Arcos Organics (New Jersey, USA, Geel, Belgium) and was dissolved in methanol or PBS solution. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, PBS, potassium persulfate and phosphate buffer was obtained from (HiMedia, Mumbai, India). A 0,15 mmol/L DPPH solution was prepared daily in methanol (Sigma-Aldrisch, Missouri, USA).

Plant material and ethanolic extraction procedure

Seeds of *E.vesicaria longirostris* were collected from places where the plant grows spontaneously in Tunisia (Kasserine, Kairouan and Sousse) and were used for the later cultivation of the plant. Samples of adult leaves of *E.vesicaria longirostris* were cultivated in (National Institute for Rural Engineering Research Water and Forests, INGREF) in Tunis. Samples were stored in a freezer at -20 ◦ C until drying and extraction. Leaves were dried with different temperatures and durations using an oven with adjustable temperature (Memmert, Schwabach, Germany).

The powder of leaves dried (10 g) were extracted with 100 mL ethanol (80:20, v/v) in a universal shaker (IKA Labor Technik HS 250 Basic, STAUFEN, Germany) during 72 hours [12-14]. In all experiments, the extractions were done in colored glass bottles of 250 mL and were stopped by a rapid filtration through a Büchner funnel (BÜCHI, Switzerland). After filtration, the extract was concentrated to dryness under reduced pressure in a rotary evaporator at 40°C (Rotavapor R II - BÜCHI, Switzerland) to yield dried ethanolic extract. The dried etanolic extract was used to prepare solutions at different concentrations for determination of total polyphenol content (TPC), determination of total flavonoids concentration (TFC) and tests of antioxidant activity.

Experimental design

To investigate the effects of temperature and duration of drying on the total phenols content (TPC), total flavonoids content (TFC) and antioxidant activity tested by DPPH essay ($DPPH^{\cdot}$), and ABTS essay ($ABTS^{\cdot+}$), an experimental design was formulated based on the Design-Expert software (version 9.0.3.1) using the 2^3 factorials. Data and results were analyzed using the Response Surface Methodology (RSM), a useful technique for process optimization. The parameter levels were chosen according to the results of previous work presented by Ioannou., *et al*. [15] and some preliminary tests

(Table 1). In the RSM analysis, 11 experiments were realized (Table 2). In this study, heat treatments are carried out by stoving. Four parameters are measured for each experiment: Total phenols content, Total flavonoids content, The antiradical activity by the DPPH test and the antiradical activity by the ABTS + test.

Parameters	Units	Coded levels		
		-1	0	1
		Original variables		
Time	min	10	20	30
Temperature	(°C)	40	70	100

Table 1: Code and Original Variables in 2^3 Factorials Design.

Parameters		
Experiences	Time	Temperature
1	- 1	- 1
2	0	- 1
3	+ 1	- 1
4	- 1	0
5	0	0
6	0	0
7	0	0
8	+ 1	0
9	- 1	+ 1
10	0	+ 1
11	+ 1	+ 1

Table 2: Matrix of experiments to be carried out for the RSM analysis.

Determination of the optimum conditions and validation of the model

The optimum conditions of extraction were looked for by using the simplex method, a general function optimization technique [16]. The Design-Expert software (version 9.0.3.1) was set to search the optimum desirability of the response variables, i.e., the maximum yield of TPC and TFC and the minimum value of CE50 for ($DPPH^{\cdot}$) and ($ABTS^{+\cdot}$). The verification of the validity and adequacy of the predictive extraction model was realized in these optimum conditions of solvent composition, temperature and time of contact. Four experimental replicates were performed at the optimized, conditions and the experimental and predicted values were compared.

Analyses of the response variables

Determination of total phenolics

Determination of Total Polyphenol Content (TPC) The TPC was determined in different extracts using the Folin-Ciocalteu method of Vazquez Roncero., *et al.* [17], cited by Nakbi., *et al.* [18]. Briefly, appropriate dilutions of extracts (0,1 ml) were oxidised with the Folin-Ciocalteu reagent (10%) and the reaction was neutralized with sodium carbonate (35%).

Determination of total flavonoids content

The method used to determine flavonoids content was described by Luximon-Ramma., *et al.* [19]. 1 ml of extract was mixed with 1 ml of ($AlCl_3$, 6 H_2O) à 2%) solution. The reaction mixture was incubated at 25°C for 10 min in dark. The absorbance was measured by a UV-Vis spectrometer (Jenway 6505 UV Vis Spectrophotometer**,** Berryville, VERGINIA) at 410 nm. The flavonoid content of the extract was calculated and expressed as mg quercetin equivalent (QE)/100 g MS.

DPPH assay

The DPPH assay of plant extracts was determined according the method described by Lopes-Lutz., *et al.* [20] and modified by Alam., *et al.* [21]. To 1 ml of the extract solution (in methanol), 0.5 ml of 0.15 mM DPPH solution (in methanol) was added. The contents were mixed vigorously and allowed to ambient temperature for 30 min. The absorbance was measured at 517 nm. EC50 value (the concentration required to scavenge 50% DPPH free radicals) was calculated. The scavenging activity was estimated based on the percentage of DPPH radical scavenged according to the following formula [22]

$$P(\%) = \left(\frac{A_1 - A_2}{A_1}\right) \times 100$$

where P was percentage of DPPH radical scavenged, A1 and A2 were control absorbance (DPPH solution without extract) and sample absorbance respectively.

ABTS assay

The ABTS assay was based on the procedure described in the study of Re., *et al.* [23] cited by Du., *et al.* [24]. The solution consist-

ing of 7 mM of ABTS and 2.4 mM potassium persulfate (1:1 v/v) was reacted in the dark for twelve hours at room temperature. Then, it was mixed with PBS buffer to obtain an absorbance value 0.700 at 734 nm. One milliliter of the diluted solution was mixed with 1 ml of the extracts with different concentrations, or PBS buffer as a blank. After a 7 min reaction, the absorbance (Abs) was measured at 734 nm. The free radical scavenging capability was calculated by the equation below and expressed as the percentage of inhibition rate of free radial scavenging compared with the blank.

$$P(\%) = \left(\frac{A_1 - A_2}{A_1}\right) \times 100$$

where P was percentage of ABTS radical scavenged, A_1 and A_2 were control absorbance (ABTS solution without extract) and sample absorbance respectively.

Time (min)	Temperature (°C)	TPC (mg AGE/g of extract)
-1	-1	22,20
0	-1	18,49
1	-1	17,34
-1	0	12,66
0	0	16,46
0	0	16,49
0	0	16,45
1	0	26,03
-1	1	30,79
0	1	41,25
1	1	48,16

Table 3: Total Phenols Content of *E. vesicraia* leaves ethanolic extracts.

Results and Discussion

Effect of Temperature and Time parameters on the quantification of total phenols

The results obtained for the study of the effects of temperature and time variation on the quantification of total phenols of arugula leaf extracts are presented in table 3. These results are statistically analyzed using software Design-Expert version 9. The analysis of variance led to the results gathered in table 4 for the extracts of the leaves of *E. vesicraia*. The validity of a mathematical model is linked to the values of R2 and R2 adjusted, the closer they are to 100% the more reliable the model. In this study, the adjusted R2 and R2 values are 92.92% and 91.61% respectively. These results show that the parameters studied make it possible to explain 92% of the variations in the results of the quantification of the total phenols obtained. On the other hand, the P value of the lack of fit test, which is greater than 0.05 (significance threshold), shows that the model corresponds well to the experimental data.

The variance analysis shows that the temperature factor has a positive linear effect on the quantification of polyphenols (Table 4). In fact, the value of P is less than or equal to 0.001 which is subsequently less than 0.05 (significance threshold). This results in the fact that an increase in temperature allowed a better quantification of phenolic compounds which can be explained by an increase in the solubility of phenolic compounds [25] as well as the release of simple phenols [26]. Phenolic acids occur in plants as metabolic intermediates and also accumulate in vacuoles [27]. Heat treatment can release more bound phenolic acids from degradation of cellular constituents. Although cell wall disruption also releases oxidative and hydrolytic enzymes that can destroy antioxidants in fruits and vegetables [27], heat treatment deactivates these enzymes to prevent loss or help release phenolic acids. These results are consistent with those of [28] who showed that phenolic compounds exhibit a certain stability even at high heat treatments such as (88°C). According to these results, it can be suggested that a controlled heat treatment in terms of duration and temperature can improve the quantification of polyphenols for a plant extract. Indeed, it was shown that thermal processes in food can cause chemical and physical reactions that affect its phenolic composition, including the release of ma trix-bound phenolics, polymerization or oxidation of phenolic compounds, thermal degradation or transformation into simpler phenolic compounds [8].

The analysis of variance shows a significant linear effect of drying time on total phenols content (P values are less than 0.05) this effect is positive since the estimated coefficient is 4.31 (Tables 4 and 5). This translates into the fact that an increase in the duration of the heat treatment can allow a better quantification of the phenolic compounds. These results are consistent with those of Dewanto., *et al*. [28] who showed that total phenols present high levels at heat treatments with a duration of up to 30 min.

Source	Sum of Squares	DL	Mean of squares	F value	P value
Model	3844,39	5	768,88	70,91	< 0.0001
A-Time (min)	308,46	1	308,46	28,45	< 0.0001
B-Temperature (°C)	1823,97	1	1823,97	168,21	< 0.0001
AB	370,39	1	370,39	34,16	< 0.0001
A^2	11,52	1	11,52	1,06	0,3119
B^2	1050,40	1	1050,40	96,87	< 0.0001
Residuel	292,77	27	10,84		
Lack of fit	78,55	3	26,18	2,93	0,0539
Pure error	214,23	24	8,93		
Total (Corr.)	4137,16	32			

Table 4: Variance analysis of total phenols content results.

The results obtained are supported by numerous studies which have shown that long-term heat treatments at a low temperature can damage the structure of phenolic compounds as well as their degradation [29,30] given the existing opportunity for hydrolytic enzymes to degrade phenols [27]. Table 5 presents the estimated coefficients for the effects of the parameters studied (temperature and time) on the quantification of phenols.

Coefficient	Estimation
Constant	17,13
A-Time	4,31
B-Temperature	10,36
AB	5,56
A^2	1,23
B^2	11,76

Table 5: Coefficients of variation estimation of phenols quantification according to time and temperature.

In the model applied an interaction effect between the parameters Temperature and Time. According to tables 4 and 5, this interaction has a positive and significant effect ($p < 0.05$) on the quantification of total phenols with values of $P < 0.0001$ which is less than 0.05 and a positive estimated coefficient (Table 5) of 5.56 this is explained by the improvement in the accessibility of phenolic compounds to quantification with a high temperature and duration of heat treatment [26-28].

The variations of phenol content in function of different parameters (time and temperature) are written according to Equation (1). Graphic representation of equation (1) was presented in figures 1 and 2.

$$TPC = + 17,13 + 4,31^* A + 10,36^* B + 5,56^* AB + 1,23^* A^2 + 11,76^* B^2 \text{ (Equation 1)}$$

Where:

TPC: Total phenol content

A: Time

B: Temperature

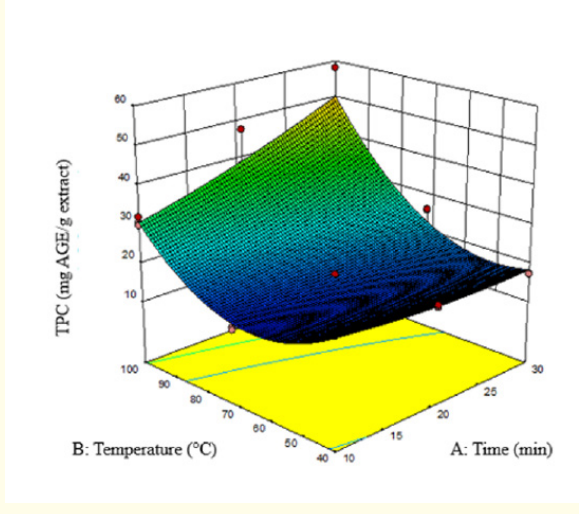

Figure 1: Total phenols content variation according to temperature and time parameters.

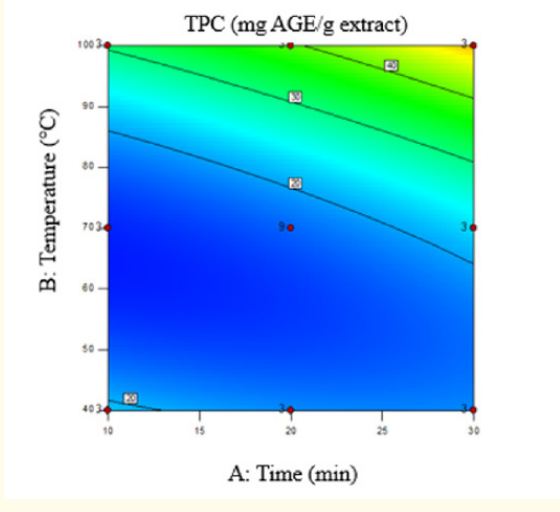

Figure 2: Representation of the estimated contours of the response surfaces of total phenols.

Effect of Temperature and Time parameters on the quantification of total flavonoids

The results obtained for the study of the temperature and time variation effects on the flavonoids quantification in ethanolic extracts of *Eruca vesicaria* leaves are presented in table 6. These results are analyzed and statistically interpreted.

Time (min)	Temperature (°C)	TFC (mg QE/g of extract)
-1	-1	15,38
0	-1	5,51
1	-1	6,04
-1	0	4,44
0	0	4,44
0	0	4,51
0	0	4,41
1	0	7,78
-1	1	10,04
0	1	15,03
1	1	19,82

Table 6: Quantification of total flavonoids from *E. vesicraia* leaves extracts.

The analysis of variance relating to the flavonoids content variation according to the parameters (time and temperature) revealed the results presented in tables 7. Table 8 presents the estimated coefficients for the effects of the parameters studied (temperature and time) on the quantification of total flavonoids.

Source	Sum of Squares	DL	Mean of squares	F value	P value
Model	860,90	5	172,18	32,19	< 0.0001
A-Time	7,08	1	7,08	1,32	0,2602
B-Temperature	161,17	1	161,17	30,13	< 0.0001
AB	274,12	1	274,12	51,24	< 0.0001
A^2	34,37	1	34,37	6,43	0,0174
B^2	300,22	1	300,22	56,12	< 0.0001
Residuel	144,43	27	5,35		
Lack of Fit	39,32	3	13,11	2,99	0,0508
Pure error	105,12	24	4,38		
Total (Corr.)	1005,34	32			

Table 7: Variance analysis of total flavonoids content results.

Coefficient	Estimation
Constant	4,27
A-Time	0,63
B-Temperature	2,99
AB	4,78
A^2	2,13
B^2	6,29

Table 8: Coefficients of variation estimation of total flavonoids content according to time and temperature.

The values of R^2 and R^2 adjusted are of the order of 85.63% and 82.97%. These results show that the parameters studied make it possible to explain 85.63% of the variations in the results of the quantification of the flavonoids obtained. The P value of the lack of fit test of the order of (0.0508) is greater than 0.05 (significance threshold), shows that the lack of fit is not significant so the model fits the data well experimental.

According to table 7, of analysis of variance, the values of P for the temperature parameter (< 0.0001) is less than 0.05 and the estimated coefficient is 2.99 (Table 7) which gives an effect on the flavonoids similar to that observed for the quantification of total phenols. An increase in temperature increases the quantification of flavonoids in the ethanolic extracts studied. These molecules are stable at high temperature and their content can increase under the effect of heat treatment. Many previous researches have demonstrated the positive correlation between temperature and flavonoids [31-34]. Some flavonoids such as quercetin and kaempferol, which exist in the extracts studied, have been shown to be very stable at high temperatures (above 100°C) and even for long heating times [33,35] showed that heating for 10, 70, 130 min at 150°C increases the rutin content which also exists in leaf samples. Nevertheless, the stability of flavonoids can be affected when the heat treatment time is very long even at low temperature [36]. showed that drying in a dryer at 45°C for 5 hours reduced the level of flavanoids kaempferol, catechin and rutin was the most stable.

The value of P obtained for the time parameter is around 0.2602, which indicates that the time parameter does not have a significant effect on the quantification of flavonoids (Table 7). Its estimated coefficient is 0.63 (Table 8), which gives a non-significant positive effect for a threshold of 0.05. An increase in the duration of the heat

treatment does not induce any effect on the flavonoid content for the extracts studied. [28] showed that the flavonoid content can remain stable with heat treatment at 88°C for a period ranging from 2 to 30 min. Dong., *et al*. (2011) also showed that the duration of the heat treatment does not influence the flavonoid content.

The P values recorded for the Time/Temperature interaction effect is < 0.0001, (Table 7), which shows a significant effect. The estimated coefficient, which is 4.78, shows a positive effect of this interaction on the quantification of flavonoids (Table 8). These results are consistent with those of Murakami., *et al*. [32] who showed that flavonoids are generally stable at high temperature 180°C for a duration of 180 min than for a duration of 120 min.

The variations of total flavonoids content in function of different parameters (time and temperature) are written according to Equation (2). Graphic representation of equation (2) was presented in figures 3 and 4.

$TFC = + 4,27 + 0,63^* A + 2,99^* B + 4,78^* AB + 2,13^* A^2 + 6,29^* B^2$ (Equation 2)

Where:

TPC: Total phenol content

A: Time

B: Temperature

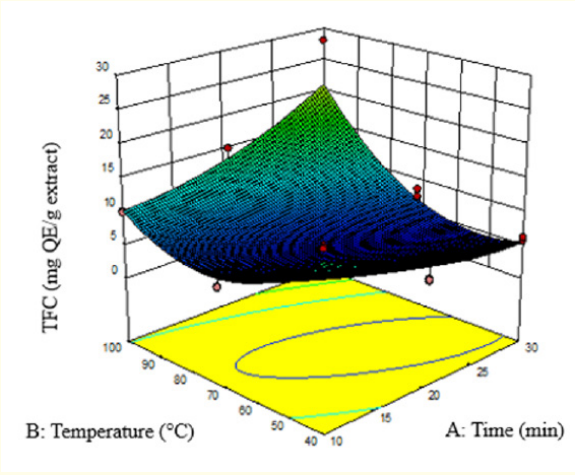

Figure 3: Total flavonoids content variation according to temperature and time parameters.

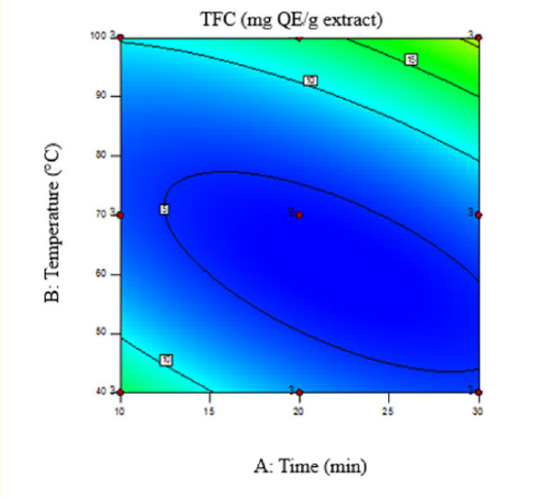

Figure 4: Representation of the estimated contours of the response surfaces of total flavonoids.

Effect of Temperature and Time parameters on the inhibition of the DPPH radical and the ABTS cation radical by the extracts of the leaves of *E. vesicaria*

The results obtained for the study of the effects of temperature and time variation on the inhibition of the free radical DPPH in the presence of extracts from the leaves of E. vesicaria are presented in table 9. These results are analyzed and interpreted statistically by the Design-Expert software version 9.

Temps (min)	Temperature (°C)	DPPH EC_{50} (µg/ml)	ABTS EC_{50} (µg/ml)
-1	-1	233,33	195,67
0	-1	471,00	361,00
1	-1	467,67	357,33
-1	0	440,67	330,67
0	0	616,00	506,00
0	0	607,50	497,50
0	0	621,50	511,50
1	0	421,00	311,00
-1	1	256,00	146,00
0	1	362,67	246,33
1	1	66,00	75,33

Table 9: Inhibition percentage of DPPH assay of *E.vesicraia* leaves ethanolic extract.

Tables 10 and 11 group together the results of the analysis of variance relating to the variation in the percentages of inhibition as a function of the parameters studied respectively for the radical DPPH and the radical cation ABTS.

Source	Sum of Squares	DL	Mean of squares	F value	P value
Model	9,189E+005	5	1,838E+005	93,58	< 0.0001
A-Time	304,22	1	304,22	0,15	0,6970
B-Temperature	1,187E+005	1	1,187E+005	60,46	< 0.0001
AB	1,350E+005	1	1,350E+005	68,76	< 0.0001
A^2	2,249E+005	1	2,249E+005	114,51	< 0.0001
B^2	2,630E+005	1	2,630E+005	133,91	< 0.0001
Residuel	53025,81	27	1963,92		
Lack of fit	9347,65	3	3115,88	1,71	0,1912
Pure error	43678,17	24	1819,92		
Total (Corr.)	9,720E+005	32			

Table 10: Variance analysis of inhibition percentage of DPPH assay of *E.vesicraia* leaves ethanolic extract.

Source	Sum of squares	DL	Mean of squares	F value	P value
Model	6,335E + 005	5	1,267E + 005	48,79	< 0.0001
A-Time	2544,22	1	2544,22	0,98	0,3311
B-Temperature	99606,72	1	99606,72	38,35	< 0.0001
AB	40484,08	1	40484,08	15,59	0,0005
A^2	1,602 + 005	1	1,602E + 005	61,68	< 0.0001
B^2	2,003E + 005	1	2,003E + 005	77,13	< 0.0001
Rasidual	70118,52	27	2596,98		
Lack of fit	17263,68	3	5754,56	2,61	0,0745
Pur Error	52854,83	24	2202,28		
Total (Corr.)	7,037E+005	32			

Table 11: Variance analysis of inhibition percentage of ABTS assay of *E.vesicraia* leaves ethanolic extract..

Table 12 presents the estimated coefficients for the effects of the parameters studied (temperature and time) on the variation in the percentages of inhibition of DPPH and ABTS radicals in the presence of extracts of *E. vesicraia* leaves. The values of R^2 and R^2 adjusted for DPPH and ABTS are respectively (94.54%; 93.53%) and (90.04%; 88.19%). These results show that the parameters studied make it possible to explain 93% and 90% of the variations in the results of the antiradical activity respectively on DPPH and ABTS +. for the leaf extracts studied. The P values of the lack of fit test, which are of the order of 0.1912 and 0.0745 higher than the significance level (0.05), indicate that the lack of fit is not significant so the model corresponds well to the experimental data.

Coefficient	Estimation	
	DPPH·	$ABTS^{+\cdot}$
Constante	610,14	489,40
A-Temps	4,11	11,89
B-Température	-81,22	-74,39
AB	-106,08	-58,08
A^2	-172,02	-145,18
B^2	-186,02	-162,34

Table 12: Regression coefficients estimation of the variation of percentages of inhibition against DPPH and ABTS radicals of leaves of *E. vesicraia* extracts.

The P values (Tables 10 and 11) relating to the change in percentage inhibition of DPPH are < 0.0001 and therefore are less than 0.05. The coefficients estimated respectively for the EC50 of the DPPH. and $ABTS^{\cdot+}$ are -81.22 and -74.39 (Table 12).

These different values indicate a significant negative linear effect of temperature on the antioxidant activity of the extracts. This shows that antioxidant activity increases with temperature. These results were supported by the work of Jeong., *et al*. (2004), who showed that the antioxidant activity increases by 50% with heating at 50, 100, 150°C for 60 min. Erbay., *et al.* [37] reported that according to the heat intense and type of the product, sometimes the antioxidant capacity can decrease (esp. foods that have high ascorbic acid content) or sometimes antioxidant properties of naturally occurring antioxidants can be improved and this seems to be related to the presence of polyphenols and their antioxidant properties which may change as a consequence of their oxidation state. In other hand, previous research has shown that the scavenging activity of phenolic compounds on DPPH increases with high temperature heat treatments [38,39]. Nevertheless, long-term heat treatment

can decrease antioxidant activity even at low temperatures [40]. Similar results were shown that the intense of heat was important and the moderate temperature was desirable to increase the retention of the antioxidant capacity of olive leaves during drying [37].

The values of P recorded for the quadratic effect of temperature (< 0.0001) are less than 0.05 and the estimated coefficients of the variation of the percentages of inhibition of the radicals DPPH and ABTS (Tables 10 and 11) are respectively - 172.02 and -145.18. These results show a significant negative quadratic effect which indicates that the antioxidant activity is limited by the temperature and beyond a well determined temperature the antioxidant activity is no longer maintained and it can decrease.

Predicting the evolution of antioxidant activity according to heat treatments is difficult given the large number of factors involved in its evolution. A decrease in phenol content will not always lead to a decrease in antioxidant activity. Indeed, the high temperature degradation products of phenolic compounds, resulting from the Maillard reaction, can have an antioxidant activity as important as the phenols themselves [32,41]. Thus, an increase in antioxidant activity is noticed in many studies using thermal processes [42,43]. On the other hand, heat treatment can release more bound phenolic acids from the degradation of cellular constituents. Indeed, [44] show that free phenolic acids have greater antioxidant activity than bound ones. However, there are important interaction phenomena that act on the antioxidant activity of molecules such as synergies between antioxidant compounds [45,46]. In some cases, antioxidant activity in a food matrix is enhanced [28,47], while in other cases antioxidant capacity is reduced [48] while in other studies the antioxidant activity remains constant [49].

The P values obtained (Table 10, 11) for the time parameter are respectively for the DPPH test. and $ABTS^{\cdot+}$ 0.6970 and 0.3311 greater than 0.05 and their estimated coefficients are respectively 4.11 and 11.89, which gives a non-significant positive effect for a threshold of 0.05. An increase in the duration of the heat treatment does not induce any effect on the antioxidant activity for the extracts studied.

The P values obtained for the Time/Temperature interaction for the antiradical activity on DPPH and $ABTS^{+}$ are less than 0.05 and their estimated coefficients are -106.08; -58.08. This shows a negative and significant linear effect of this interaction. Knowing that the effect of time is not significant, the effect of the interaction of the two parameters follows the trend of the results of the effect of temperature. Indeed, antioxidant activity increases with temperature. This phenomenon has been observed in many previous studies [42,43]. These results are correlated with those obtained for total phenols and flavonoids.

According to table 12, two equations are deduced describing the variation in the percentages of inhibition of the DPPH radical and of ABTS under the effect of the parameters studied (Equation 3 and 4). Graphic representation of equation (3) and (4) was presented in figures 5 and 6.

DPPH = + 610,14 + 4,11* A-81,22* B-106,08* AB-172,02* A^2-186,02* B^2 (Equation 3)

ABTS = + 489,40 + 11,89* A-74,39* B-58,08* AB-145,18* A^2-162,34* B^2 (Equation 4)

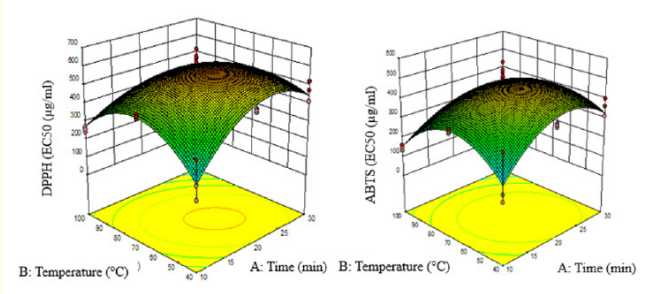

Figure 5: Variation of DPPH inhibition percentages. and $ABTS^{\cdot+}$ of leaves ethanolic extracts according to parameters (temperature and time).

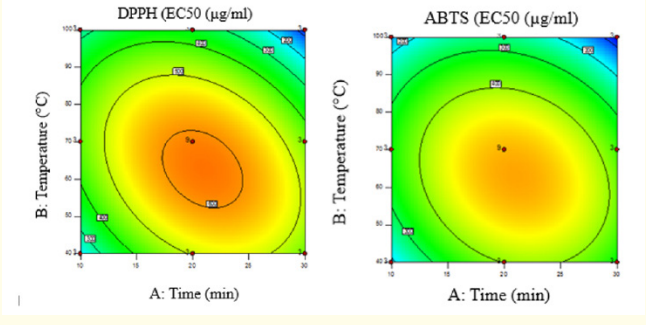

Figure 6: Estimated response surface contours graphic representation of percentage inhibition against DPPH and ABTS of ethanolic extracts according to temperature and time parameters.

Determination of optimal conditions for heat treatment of *Eruca vesicaria* leaves

The optimization by Design-Expert software version 9 of the results obtained for the study of the effects of temperature and time variation on the quantification of phenolic compounds and the antioxidant activity of ethanolic extracts from the leaves of the plant *E. vesicaria* was realized (Table 13). In this study, the optimization was applied for selected ranges of temperature and process time as 40-100 °C, and 10-30 min, respectively. By applying desirability function method, four solutions were obtained for the optimum covering criteria with desirability value ranged from 0,666 to 1,7. All were close to each other such as temperatures and process times varied between 96.34-100.00°C and 29.01- 30.00 min, respectively. Similar results were reported by Erbay and Icier [37]. The optimal heat treatment conditions (100°C for 30 min), selected by the Design-Expert statistical software, present the best desirability value of around 0.767. The conformity of the experimental values with those predicted by the mathematical models confirms the choice of the optimum 100°C for 30 min (Table 13).

	Time	Temperature	TFC	TPC	DPPH	ABTS	DesirabilitY
Predicted values	30,00	100,00	21,08	50,35	68,91	61,30	0,767
	30,00	99,75	20,91	50,07	73,57	65,11	0,760
	29,01	100,00	20,14	49,14	111,31	93,15	0,734
	30,00	96,34	18,69	45,70	134,47	114,73	0,666
Observed Value	30,000	100,000	19,82	48,16	66,00	75,33	

Table 13: Optimal heat treatment conditions.

Conclusion

From this study, it can be concluded that, the increase in the duration and temperature of the heat treatment can allow a better quantification of the phenolic compounds. Results indicate the antioxidant activity increases with temperature. Results showed a negative and significant linear effect of Time/Temperature interaction for the antiradical activity on DPPH and $ABTS^{+}$. In this study fore models are obtained to represent the evaluation of TPC, TFC and antioxidant activity according heat treatment conditions (time and temperature of drying). The optimum drying parameters (100°C for 30 min) leading to better quantification of phenolic compounds and better antioxidant activity in ethanolic extracts of *E.vesicaria* leaves. This paper shows that leaves of *E.vesicaria longirostris* plant which grows spontaneously in Tunisia with health-promoting effects and spicy hot taste can be used as drayed spice use.

Conflict of Interest

Financial interest or conflict of interest are not existing.

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