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Application of response surface methodology to optimize the extraction of essential oil from *Rosmarinus officinalis* using microwave-assisted hydrodistillation

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ABSTRACT

The present study was conducted to optimize the microwave-assisted hydrodistillation process for the extraction of essential oils from Moroccan rosemary using response surface methodology. In this methodology, four independent variables were estimated by means of a central composite design in relation to essential oil yields, mainly extraction time (20, 55, and 90 minutes), microwave power (200, 400, and 600 W), water-to-plant ratio (2, 4, and 6 ml/g), and drying period (0, 7, and 14 days). The extraction yield according to the mathematical regression model correlation analysis was expressed using a second-order polynomial. The maximum essential oils' yield was found to be 1.326% at the optimum conditions of 20 minutes, 600 W, 2 ml/g, and 7 days.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.), as a perennial herb belonging to Lamiaceae family, is utilized in cosmetics, in traditional medicine, and as a food preservative and flavoring agent (Ramírez *et al*., 2006). This plant has high antioxidant activity, antimicrobial, and antimutagenic characteristics, and is also known as a chemopreventive agent (Ibañez *et al*., 2003; Oluwatuyi *et al*., 2004). Rosemary has been found to have abundant essential oil in the flower and in the leaves. While oil extraction can be done from both locations, the leaves containing essential oil glands have the most high-quality essential oil (Carvalho *et al*., 2005).

Because of its utilization in several applications, such as food preservatives, cosmetics, and pharmaceutical medicines, extracting essential oils from aromatic plants is a highly essential research topic. A variety of oil extraction methods are applied for medicinal plants, for instance, rosemary plant, including steam distillation, percolation, hydrodistillation (HD), supercritical fluid extraction, and ultrasound and microwave-assisted extractions (Belhachat *et al*., 2018; Presti *et al*., 2005).

Extracting essential oils by microwave-assisted hydrodistillation (MHD) is more utilized in both laboratories and industries because of many features, such as rapid energy transfer and efficient heating, in addition to the environmentally eco-friendly isolation system (Filly *et al*., 2014). The MHD in comparison with other conventional methods of extraction, such as HD, shows high performance in terms of improvement of quality, as well as quantity of the isolated oils, reduction for the extraction time, and reduction in both cost and energy consumption, as well as the minimization of the carbon dioxide quantity emitted into the atmosphere (Elyemni *et al*., 2019; Karakaya *et al*., 2014; Moradi *et al*., 2018).

Generally, MHD extraction efficiency can be affected by several variables, alone or in combination, which includes

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extracting time, water-to-plant ratio, the microwave power supply, and drying time (Benmoussa *et al*., 2018; Mathialagan *et al*., 2014; Turk *et al*., 2018). Hence, it is necessary to optimize these parameters to obtain a higher yield.

Traditionally, the optimization studies are conducted using a classical optimization method called one-variable-at-a-time, where one variable changes at a time but others remain unchanged (Kannan *et al*., 2004). In such a method, the main drawback is that the excluded interactive influences found between variables and experimental work required are increased, leading to increased time usage, cost, and use of reagents and materials (Bezerra *et al*., 2008).

Response surface methodology (RSM), as a solution to the above-mentioned issue, is highly recommended because of its ability to optimize experimental conditions for a multivariable system, while decreasing the number of needed experimental trials for evaluating several parameters and their interactions. RSM is useful in different methods, including central composite design (CCD) and Box–Behnken design, in addition to a three-level full factorial design (Baş and Boyacı, 2007; Montgomery, 2017). Recently, RSM has been successfully employed in chemistry, biology, food, agriculture, engineering, and other research fields (Bashir *et al*., 2010; Hasani *et al*., 2019; Sharma *et al*., 2018; Sodeifian *et al*., 2018).

In our work, we aim to employ RSM in terms of exploration as well as optimization of several variables, such as the extraction time, water-to-plant ratios, microwave power, and finally the drying period for extraction of rosemary essential oil by MHD.

MATERIALS AND METHODS

Plant material

Freshly harvested rosemary aerial parts were realized at the stage of flowering in May 2018, located in Fez region. The dried plant was attained when left in the shade at 25°C for 7 or 14 days.

Microwave-assisted hydrodistillation

The MHD was accomplished with the assistance of a microwave oven as a power supply, with specifications of MWD 119 WH, whirlpool, China, 20 L, 2.45 GHz, that had a direct connection with a Clevenger appliance as well as a cooling system for continuous condensation of the distillate. The microwave oven has a power consumption of 1,100 Watts and an output power of 700 Watts, with a power source of 230 v–50 Hz with cavity dimensions of 216 × 302 × 277 mm.

In the MHD procedure, 100 g of fresh or dried rosemary samples was mixed with various volumes of water (200, 400, and 600 ml) in a flask (2 l) and heated inside the microwave oven cavity at various powers (200–600 W) and various extraction times (20–90 minutes). The vapor mixture of water and essential oil was condensed continuously within a cooling system at the exterior of the microwave cavity and recovery was attained in a Clevenger receiving. The excess condensed water was refluxed into the flask of extraction for providing unvarying environments of humidity for the extraction process (Elyemni *et al*., 2019). Subsequently, the essential oils collected were charged with anhydrous sodium sulfate to be dehydrated, weighed, and finally stored at 4°C in a vial in the dark.

Experimental design of RSM and statistical analysis

To define the optimum conditions, a three-level-four-factor CCD, followed by RSM, was utilized, affording the highest yield of the extracted essential oil derived from rosemary and aided by the MHD. The independent variables studied were extracting time, microwave power supply, water-to-plant ratio, and drying period, whereas the essential oil extraction yield was the response variable, which is calculated by using:

$$\text{Yield (\%)} = \frac{\text{Amount of extracted essential oil (g)}}{\text{Amount of vegetal matter (g)}} \times 100 \qquad (1)$$

The central point and range of the four variables had been chosen depending on the preliminary experiments' outcomes. In each parameter, three levels were examined and have different codes as follows: –1 for lower level, +1 for higher level, and 0 for a central coded value as shown in Table 1.

According to CCD, 30 experimental runs were proposed that comprise 16 factorial runs and 8 axial runs in addition to 6 replicate runs at the center. The center point replicates can provide a pure error estimate, and experiments were conducted randomly to prevent systematic error. The experimental design in addition to statistical analysis was achieved by Design Expert 11 Trial. The response employed for establishing an empirical model that linked response to four input variables by means of a second-degree polynomial is displayed as:

$$Y = \beta_0 + \beta_{ii} X_i + \beta_{ii} X_i^2 + \beta_{ij} X_i X_j + \varepsilon \qquad (2)$$

where Y represents the response function, β_0 is the intercept term, X_i and X_j are coded independent parameters, β_i are linear coefficients, β_{ii} are quadratic coefficients, β_{ij} are interaction coefficients, and ε is the experimental error (Benmoussa *et al*., 2018; Mathialagan *et al*., 2014).

Statistical examination of the model was realized using the analysis of variance (ANOVA) analysis as well as the F-test for exploring the ideal relationship between input factors and extraction yield. Each term's significance in the equation of the model was evaluated statistically when the corresponding p-values (significance confirmed if $p \leq 0.05$) were calculated (Ara and Raofie, 2016; Rai *et al*., 2016). For evaluating the model reliability, coefficient of determination (R^2), adjusted R^2, predicted R^2, lack of fit, and adequate precision were used. The response surfaces and contours plots were created for determining the individual test variables or interactive effects on essential oil yield and to deduce the optimum conditions.

Table 1. Experimental variables and their levels.

Factor	Levels		
	−1	0	+1
A: extraction time (minutes)	20	55	90
B: microwave power (W)	200	400	600
C: water-to-plant material ratio (ml/g)	2	4	6
D: drying period (days)	0	7	14

RESULTS AND DISCUSSION

The experimental design together with the experimental extraction values in the presence of various combined extraction settings is presented in Table 2.

Response ranges from 0.034% to 1.331% according to the parameters of the experiments. The maximum yield achieved at MHD is as follows: time of 55 minutes, a microwave power of 600 W, water-to-plant material ratio of 4 ml/g, and drying time of 7 days (run 18).

Table 2. Experimental design matrix and the extraction yield evaluated at various experimental settings.

Run	Factors				Extraction yield (%)
	A (minutes)	B (W)	C (ml/g)	D (days)	
1	90	400	4	7	1.316
2	20	200	2	0	0.182
3	20	600	6	14	1.017
4	55	400	2	7	1.255
5	20	600	2	14	1.107
6	55	400	4	7	1.114
7	90	600	2	14	1.133
8	20	200	2	14	0.397
9	55	400	6	7	1.061
10	20	400	4	7	0.968
11	55	400	4	14	0.971
12	55	400	4	7	1.055
13	55	400	4	7	1.109
14	90	600	6	14	1.025
15	20	600	2	0	0.867
16	20	200	6	0	0.034
17	90	600	6	0	0.649
18	55	600	4	7	1.331
19	90	200	2	14	1.042
20	55	400	4	7	1.034
21	55	200	4	7	0.903
22	90	600	2	0	0.870
23	20	600	6	0	0.607
24	20	200	6	14	0.408
25	55	400	4	7	1.073
26	90	200	6	0	0.566
27	90	200	2	0	0.755
28	55	400	4	7	1.084
29	55	400	4	0	0.630
30	90	200	6	14	0.889

Model fitting and ANOVA

The four most widely employed models of linear, two-factor interaction (2FI), quadratic, and cubic models are assessed in accordance with the scores generated by the sequential sum of squares model, and many statistics to evaluate their adequacy are summarized in Table 3 and 4. Compared to other models, the optimal model identified is the quadratic model with the largest F-value of 91.81 and the smallest value of $p < 0.0001$ (Table 5). Besides, the nonsignificant value of the lack of fit (p-value of $0.1130 > 0.05$) for the quadratic model revealed the validity of this model, with better credibility and accuracy (Qi *et al*., 2014).

The equation for the second-order polynomial regression is presented as follows, regarding the coded factors obtained from the software:

$$\text{Yield (\%)} = 1.11 + 0.1477A + 0.1906B - 0.0751C + 0.1572D - 0.1345AB - 0.0115AC + 0.0006AD - 0.0125BC + 0.0056BD + 0.0299CD - 0.0095A^2 - 0.0345B^2 + 0.0065C^2 - 0.3510D^2$$

The coefficients' sign and magnitude allow the interpretation of the variable effects on the response. The quadratic model showed a positive impact on the yield extracted for the linear variables A, B, and D, the interactions of the variables AD, BD, and CD, and the variable quadratic C^2, while the variables C, AB, AC, and BC and the quadratic variables A^2, B^2, and C^2 exhibited adverse consequences.

As Table 3 shows, the ANOVA of the quadratic polynomial model selected for MHD of rosemary revealed R^2 determination coefficient as of 0.9885, signifying the ability of the model to explain 98.85% of the data variation, while it has no explanation for only 1.15% of the total variations.

The adjusted R^2 (0.9777) value was precisely close to its corresponding R^2, which suggests a strong correlation of the observed and anticipated data. The difference between the adjusted and predicted R^2 should therefore be less than 0.2 to be in judicious matching (Owolabi *et al*., 2018; Rai *et al*., 2016). This requirement is satisfied with a predicted R^2 value of 0.9564.

The data dispersion is described by the coefficient of variation (CV), where reproducibility with better values was achieved at lower values of CV% (< 10%) (Zhang *et al*., 2014). For the suggested models, such value is 4.35% denoting high

Table 3. Sequential model sum of squares for MHD of rosemary.

Source	Sum of squares	df	Mean square	F-value	p-value	
Mean *vs.* total	23.32	1	23.32			
Linear *vs.* mean	1.59	4	0.3981	7.23	0.0005	
2FI *vs.* linear	0.3089	6	0.0515	0.9156	0.5051	
Quadratic *vs.* 2FI	1.03	4	0.2585	113.19	< 0.0001	Suggested
Cubic *vs.* quadratic	0.0100	8	0.0013	0.3628	0.9106	Aliased
Residual	0.0242	7	0.0035			
Total	26.29	30	0.8764			

Table 4. Summary of model statistics for MHD of rosemary.

Source	Std. dev.	Sequential *p*-value	Lack of fit *p*-value	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.2347	0.0005	< 0.0001	0.5363	0.4621	0.3056	2.06	
2FI	0.2371	0.5051	< 0.0001	0.6403	0.4509	−0.2804	3.80	
Quadratic	0.0478	< 0.0001	0.1130	0.9885	0.9777	0.9564	0.1295	Suggested
Cubic	0.0588	0.9106	0.0173	0.9918	0.9662	0.2085	2.35	Aliased

Table 5. Statistical analysis of variance of the quadratic model generated from CCD for rosemary essential oil extraction yields.

Source	**Sum of squares**	**df**	**Mean square**	***F*-value**	***p*-value**	
Model	2.94	14	0.2096	91.81	< 0.0001	Significant
(A) Extraction time	0.3925	1	0.3925	171.88	< 0.0001	
(B) Microwave power level	0.6536	1	0.6536	286.22	< 0.0001	
(C) Water-to-plant material ratio	0.1016	1	0.1016	44.47	< 0.0001	
(D) Drying time	0.4446	1	0.4446	194.71	< 0.0001	
AB	0.2894	1	0.2894	126.75	< 0.0001	
AC	0.0021	1	0.0021	0.9266	0.3510	
AD	6.250E-06	1	6.250E-06	0.0027	0.9590	
BC	0.0025	1	0.0025	1.09	0.3120	
BD	0.0005	1	0.0005	0.2217	0.6445	
CD	0.0143	1	0.0143	6.25	0.0245	
A^2	0.0002	1	0.0002	0.1024	0.7534	
B^2	0.0031	1	0.0031	1.35	0.2634	
C^2	0.0001	1	0.0001	0.0479	0.8296	
D^2	0.3192	1	0.3192	139.78	< 0.0001	
Residual	0.0343	15	0.0023			
Lack of fit	0.0295	10	0.0029	3.08	0.1130	Not significant
Pure error	0.0048	5	0.0010			
Cor. total	2.97	29				
Fit statistics for regression analysis						
Std. dev.	Mean	CV%	R^2	Adjusted R^2	Predicted R^2	Adeq. precision
0.0478	0.8817	5.42	0.9885	0.9777	0.9564	36.9686

precision and reliability (Table 5). Finally, the value of "adequate precision" of 35.2024 is greater than 4, suggesting an appropriate signal-to-noise ratio (Hu *et al*., 2018).

The *p*- and *F*-values were utilized as an evaluating tool for each coefficient significance of the quadratic model (Table 5), where the smaller the *p*-value and the higher the *F*-value, the more important the respective coefficient. In the current work, the significant classification of the factors is B > D > A > D^2 > AB > C > CD, while the other coefficients do not affect the extraction yield significantly ($p > 0.05$). Hence, after ignoring the insignificant terms, the final anticipated polynomial second-order equation attained is given by:

$$\text{Yield (\%)} = 1.10 + 0.1477A + 0.19067B - 0.0751C + 0.1572D - 0.1345AB + 0.0299 - 0.3781D^{2.}$$

Adequacy of the second-order polynomial models

Three diagnostic plots can confirm the fitted model adequacy, including predicted versus actual plots (Fig. 1a), the normal probability plot against the residual values (Fig. 1b), and the residuals plot against the run number (Fig. 1c).

Figure 1a shows that the values based on the experimental work in comparison with the expected ones were close to being a straight line, offering a well-matched pattern for the determined as well as anticipated values. Such a result supported the least square fit adequacy.

A normal probability plot was generated to check the normality of the internally studentized residual. The proximity of point distributions along the straight lines shows that the residuals for essential oil responses were typically distributed and that the fitted model provided a reasonable estimate to rosemary essential oil's experimental yields.

The last plot of residuals for rosemary essential oil's yield against the experimental run order was designed for investigating the practical response and its satisfactory fit (Benmoussa *et al*., 2018). The diagnostic plot demonstrates that within certain limits (±3) all the data points were distributed unsystematically.

Response surfaces and contour plots analysis

For visualizing the independent variables' effect in addition to their collaborative interactions on the rosemary extraction yield, three-dimensional response surface and two-

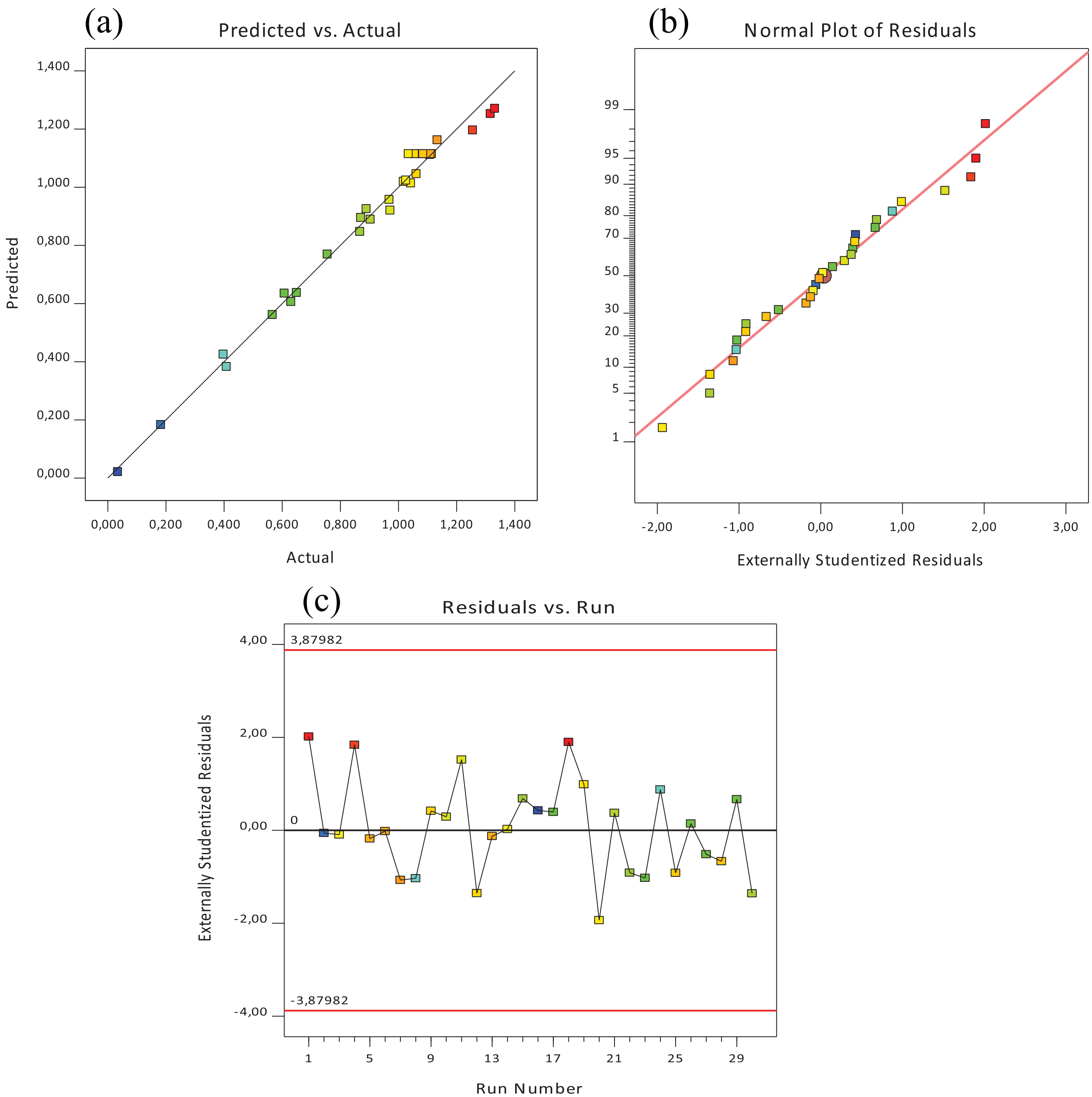

Figure 1. Diagnostic plots of model adequacy. Predicted versus actual plots (a), normal probability plots of residuals (b), and the residuals versus the run number (c).

dimensional contour plots were built. Two parameters were kept at their central level values coding at a zero, and two different parameters were used for realizing the individual and interactive effect of their response. Response surface plot of extraction yield as a function of extraction time and microwave power (Fig. 2) indicates that the yield of essential oil was positively influenced by the microwave power. Furthermore, at a definite microwave power, the yield of extraction increases with increasing extraction time.

The improved extraction yield based on increased time of extraction is attributed to the microwave adsorption energy in addition to the interfacial area between the plant matrixes and solvent, which enhances the essential oil dissolution process into the water (Mollaei *et al.*, 2019).

The power of microwaves serves as a driving force to wreck the plant cell membrane structure, which causes the diffusion and the dissolution of the oil in the solvent. Microwave power also has a direct influence on temperature, in which a higher microwave power increases the temperature of the distillation due to the increase in dielectric heating phenomena. Consequently, increased power will usually increase yield and speed up the extraction time (Benmoussa *et al*., 2018; Chen *et al*., 2016). The yielded findings are well-matched with those of Mathialagan *et al*. (2014), who found the equivalent consequence of the microwave irradiation power as well as time on the extracted essential oil yield from the leaves of lemongrass (*Cymbopogon citratus*) when MHD was employed. Likewise, an extracted essential oil based on *Ferulago angulata* fruit was realized by microwave-aided hydrodistillation and achieved an equal result described by Mollaei *et al*. (2019).

Figure 3 shows the influence of the plant-material-to-solvent ratio and drying period on the extraction rosemary essential oil yield at a fixed microwave power of 400 W and fixed extraction time of 40 minutes. The extracted yield decreases slightly with the increase in the plant-material-to-solvent ratio at a constant drying period. This decline was also reached by Abdelhadi *et al*. (2015) and Mollaei *et al*. (2019) because of the hydrolyzed volatile ingredients in the presence of high water content. Also, at fixed plant-material-to-solvent ratio and drying period affected the essential oil yield in a quadratic manner. So, low plant-material-to-solvent ratio and moderate drying period were more suitable for the essential oil yield.

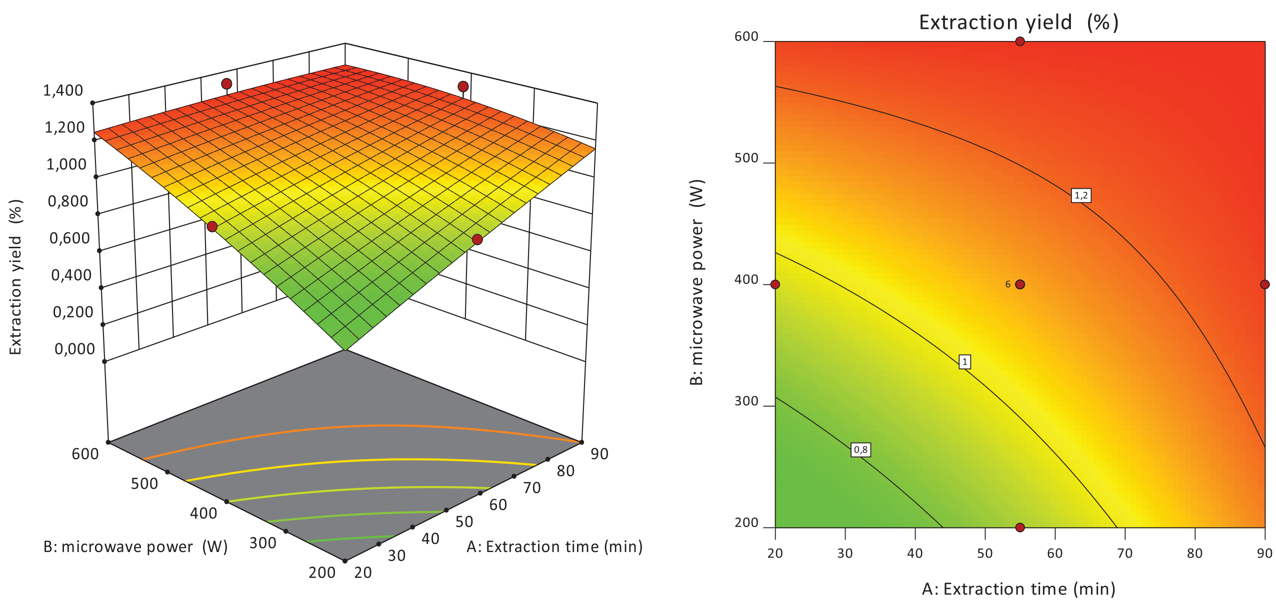

Figure 2. 3D graphic surface and contour plot for the influence of microwave power level and extraction time on the extraction yield.

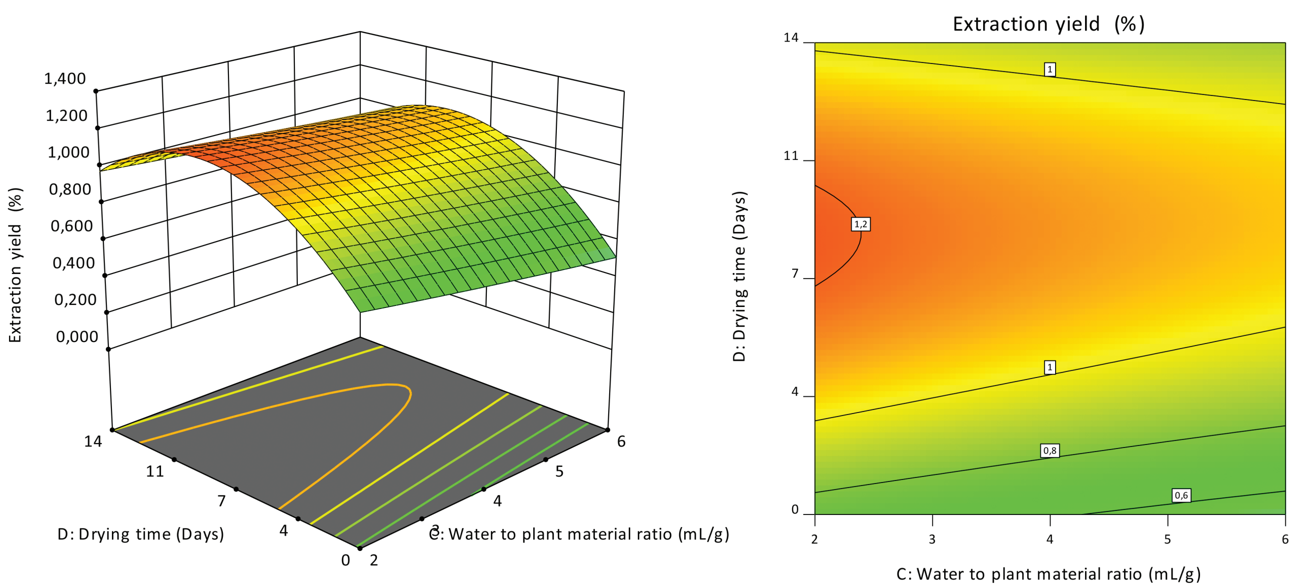

Figure 3. 3D graphic surface as well as contour plot showing microwave power level and extraction time effects on the extraction yield.

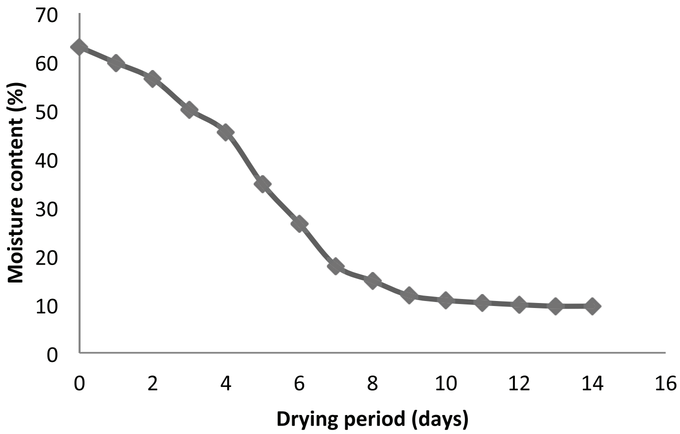

Figure 4. Evolution of the moisture content of *R. officinalis* leaves during the drying period.

To better explain the effect of drying period on the rosemary essential oil yield, Figure 4 shows the evolution of moisture content according to the drying time.

Regarding the first days, the essential oils rise is proportional to the reduced humidity content, with maximum oil yields between 7 and 9 drying days. Beyond this period, the moisture content tends to stabilize while the yield decreases gradually.

Such results are in accordance with those yielded with other aromatic plants, such as *Mentha spicata* (Díaz-Maroto *et al*., 2003), *Origanum vulgare* (Novák *et al*., 2011), and *Warionia saharae* (Essaqui *et al*., 2016). The rise in the essential oils' yield using rosemary leaves can be described by a crucial physiological activity and enzymatic reactions during the first days of drying. In fact, the plant after harvest increases its biosynthetic activity of terpenes and derivatives as defense strategies against water stress. When the drying period exceeded 9 days, the essential oils' yield was decreased because of the decline or discontinuation of biosynthesis activities when the death of cells was realized after extreme dehydration. Consequently, essential oils losses by evaporation are no longer compensated.

Optimization of extraction conditions of R. officinalis essential oil

According to RSM findings, the optimum settings of MHD for maximum yield of rosemary essential oil are a microwave power of 600 W, a time of 20 minutes, a plant-matter-to-water ratio of 2 ml/g, and a drying time of 7 days. At this optimal setting, the rosemary oil yield anticipated was 1.326%. The optimized conditions have been verified experimentally and the extracted oil was identified by gas chromatography-mass spectrometry in a previous work (Elyemni *et al*., 2019).

CONCLUSION

In the current research, an RSM-based CCD was successfully employed for evaluating the influences of four independent variables, including extraction time, plant-material-to-solvent ratio, and microwave power as well as drying period on the rosemary essential oil yield, and to envisage the ideal operating settings. The experimental findings revealed that microwave power and extraction time were the most substantial parameters influencing the rosemary essential oil extraction yield. The quadratic model established for the extraction yield exhibited a well-matched pattern between the experimental data and model predictions (R^2 = 0.9885). The best conditions anticipated by the model for the designated extraction variables are as follows: power microwave (600 W), extraction time (20 minutes), water-to-plant material ratio (2 ml/g), and a drying period (7 days) with a yield of 1.326%.

CONFLICT OF INTEREST

The authors declared that they do not have any conflicts of interest.

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None.

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