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The screening of parameters influencing the hydrodistillation of Moroccan *Mentha piperita* L. leaves by experimental design methodology

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Abstract
The aim of this study was to perform a screening for the hydrodistillation process parameters in order to attain the highest possible yield of peppermint essential oil. The screening process was carried out by a Placket and Burman design. After the choice of seven variables, sixteen experiments were executed. The data analysis has permitted to identify a first-order polynomial model relating the response (essential oil yield) to the studied factors. After the validation of the fitted model, the results allowed us to conclude that five factors (the extracting time, the individuality effect, the material/water ratio, the harvest period and the heating temperature) have a significant effect on the hydrodistillation process. However, the plant material's drying and cutting present a statistically negligible effect.

Keywords: Mentha piperita L., *hydrodistillation, essential oil, Plackett & Burman design.*

1. Introduction
The particular orography of Morocco gives it a very diversified soils and climatic conditions which are favorable for the development of a rich and varied flora [1]. Being one of the most popular plants in Morocco, peppermint, *Mentha piperita* L. is an evergreen belonging to the family of *Lamiaceae* and considering among the most popular single ingredient herbal teas. Beside its uses in food, herbal tea preparations, and confectioneries, peppermint has also medicinal uses which date back to ancient times [2]. *Mentha piperita* L. essential oil has many reported benefits. Its antioxidant capacity has been determined using different assay methods [3] and its antimicrobial activity is another widely studied property [4]. Furthermore, other medicinal activities have been attributed to this oil including antitumor, antiallergenic, antiviral and fungicidal applications [5]. Therefore, it's essential to understand the effects of factors acting on its hydrodistillation process, and to find their close link with the essential oil yield's increase. To achieve this objective, we have used the experimental designs methodology which allows the obtaining of maximum information in a minimum number of experiments [6]. This method give the opportunity of classifying the factors from the most to the least important, and optimizing the operating conditions in order to obtain the best possible result.
The use of experimental designs in the analysis and the optimization of the essential oil's extraction process was reported by several authors. Some ones have used other types of screening designs such as complete factorial design [7-9], and others have performed directly the optimization by using the response surface methodology [10,11].
In this paper, we have made a screening for the factors acting on the hydrodistillation operation of *Mentha piperita* L.. Hadamard matrix and more precisely Plackett & Burman design which is the best-known screening designs for factors with 2 levels has been used [12]. The experimentation has highlighted the effects of some factors on the studied response [8]. The choice of Plackett & Burman design type instead of the complete factorial design is based on the number of the studied factors which is equal to seven. Indeed, a factorial design

will cause an increase in the number of experiments (2^7 = 128 experiments for seven factors). As for the response surface designs, these designs are generally used for the optimization and not for the screening. In our case, the objective was to determine the most important factors acting on hydrodistillation process of peppermint. Therefore, a screening design of Plackett & Burman is more advocated. In perspective, an optimization study must be carried out on the factors considered influential on the hydrodistillation process.

2. Materials and methods

2.1. Vegetable material

Mentha piperita L. plants were collected from the National Institute of Medicinal and Aromatic Plants garden in Taounate city (Morocco) (latitude 34°29'59.4"N; longitude 4°48'16.6"W).

2.2 Extraction materials

The Clevenger type apparatus was used for hydrodistillation [13] according to the method recommended by the French Pharmacopoeia [14]. The process operates at the atmospheric pressure and is equipped with a recycling system, permitting the mass plant/water ratio to be maintained at its initial level. During every experiment, plant material and water were placed, in determined proportions, in a one liter capacity glass flask. The mixture was heated to boiling temperature and the liberated steams crossed up the column and passed out of the condenser in a liquid state. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The obtained essential oil was dried over anhydrous sodium sulphate and was stored in the refrigerator at 4°C in dark glass bottles until use.

2.3 Gas Chromatography-Mass spectrometry

In Order to determine chemical composition of the essential oil chemical composition of each of studied individuals, we performed an analysis by gas chromatography coupled with mass spectroscopy. Analyses were performed by a system with electronic pressure control Hewlett Packard (HP6890 series) coupled with a mass spectrometer (HP 5973 series). The GC was equipped with fused silica capillary column (5% phenyl methyl polysiloxane), (30 m × 0.25 mm), coated with 0.25 μm film Rtx-5MS. The injection and detection temperatures were set at 250 and 280°C, respectively. The applied oven temperature program was: 40°C for 5 min, rising at 19°C/min to 280°C and held for 5 min. The control mode was split and split ratio 5:10. Carrier gas was helium with flow-rate of 1.4 ml/min. 1 µl of oil was introduced directly into the source of the MS via a transfer line with a split ratio of 1:50. The mass spectra were recorded over a range of 30 to 1000 atomic mass unit at 0.5 s/scan. Solvent cut time was 3 min. Ionization energy was 70eV. The inlet and ionization source temperature was 280°C.

2.4 Placket and Burman Design

Based on a process or phenomenon, the first problems which the experiments design can provide information about, are those of the study of a large number of factors. A screening study may be defined as a step for identifying rapidly the factors that are actually influential on a process in a fixed experimental field.

The most known matrices of screening are the matrices of Plackett & Burman [12] based on Hadamard matrices [15]. For these matrices, the number of experiments is close to the numbers of the studied factors [16]. Plackett & Burman designs are generated from matrices with orthogonal columns composed only of values +1 or -1 [17] and the mathematical model is a model without interactions [18]. The main effect (the contrast coefficient) of such design may be simply calculated as the difference between the measurement's means at the factor's high level (+1) of the measurements mean at the low level (-1). Contrast coefficients allow the determination of the effect of each factor. A large contrast coefficient either positive or negative indicates that a factor has a large impact on response while a coefficient close to zero means that a factor has little or no effect [19].

2.5 Experimental domain of factors and responses

The levels of factors were selected by taking into account the operating experimental limits, the literature data on hydrodistillation conditions [20], and the previous studies [7- 11, 21].

Factors that could affect the essential oil yield can be divided into two categories. Continuous or quantitative factors:

The extracting time varying between 150 and 210 minutes.

The ratio between the vegetable material and water. This factor varies between 1/12 and 1/4 (x 100g/100ml).

The flask's heating temperature which is directly related to the steam flow leaving the heated flask and hence the flow of condensation. To test this parameter, two heating temperatures were used: 250 °C and 350 °C.
Qualitative factors:
The harvest period of plant material having two levels: the middle of February and the middle of August.
The plant's drying with the modalities fresh plant and dried plant. The plant's drying is performed in the shade during eight days at a temperature room fixed at 25 °C.
The individual effect, this factor includes the two modalities "individual 1" and "individual 2" because we have treated two separate plants.
The cutting of plants in small pieces before the hydrodistillation which has the two modalities: entire plant and cut plant. The seven studied factors are shown in table 1.

Table 1: Parameter levels and coded values used in the experimental design

Factors	levels	Units	Coded variables	Coded levels
Harvest period	Middle of February	-	X1	-1
	Middle of August			1
Individuality effect	Individual 1	-	X2	-1
	Individual 2			1
Cutting of leaves	Cut leaves	-	X3	-1
	Entire leaves			1
Material / Water Ratio	1/12	x 100g/100ml	X4	-1
	1/4			1
Extracting time	150	Min	X5	-1
	210			1
Heating temperature	250	°C	X6	-1
	350			1
Drying	Fresh plant	-	X7	-1
	Dried plant			1

The studied response is essential oil yield of *Mentha piperita* L. expressed as:

$$Y = Y_{HE}\,(\%) = \frac{M_{HE}}{M_s} \times 100$$

Where Y_{HE}: the essential oil yield (%), M_{HE}: the essential oil mass (g) and Ms: the dry vegetal matter mass (g).

2.6 Experimental matrix
Since we have seven factors, the experimental design was a matrix of eight experiments. For more precision, we have duplicated the chosen design, which thus leads to a matrix of sixteen essays. For each experiment, the recorded response is the average of three replicates.

2.7 Mathematical model and statistical analysis
The resulting mathematical model is a first-order polynomial as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + \varepsilon$$

With:
$Y = Y_{HE}$ (%) is the yield of essential oil (response).
b_0 represents the theoretical average value of responses.
b_1, b_2, b_3, b_4, b_5, b_6 and b_7 are the principal effects of factors X_1, X_2, X3, X_4, X_5, X_6 and X_7 respectively.
ε is an error term.
An analysis of variance consisting on F test at a 95% significance level was conducted. The mean squares (MS) were obtained as follows:

$$MS = {}^{SS}/_{DF}$$

Where SS is the sum of squares of each variation source and DF is the respective degree of freedom. The ratio between the mean square regression (MS_R) and the mean square residual (MS_r), $F_{ratio(R/r)}$, was used in order to establish whether the model was statistically significant [7]. The greater F value from unity explains adequately the variation of the data around its mean, in addition the estimated factor effects are real [22, 23].
The quality of fitting the first-order polynomial was also expressed by the coefficient of determination R^2. This coefficient measures the proportion of total variation about the mean response explained by the regression. In fact, it provides information on the correlation between observed and predicted response and it is often expressed as a percentage [24].
The model coefficients were considered significant for values of *p-value* <0.05. The statistical significance of the model coefficients was determined by using the *t-test* (only significant coefficients with *p-value* < 0.05 are included). During this study, we have used the software of experiments design Nemrodw [25].

3. Results and discussion

3.1 Chemical composition of the essential oils

Twenty seven constituents representing 99.52% of the total essential oil of *Mentha piperita* L.were identified. The essential oil was characterized by a high percentage of oxygenated monoterpenes (92.95%) and low concentration of Monoterpenes hydrocarbons and sesquiterpenes hydrocarbons (5.61 % and 0.96% respectively). Tree major compounds were detected with a content higher than 10% including Menthol (46.32%), Menthofuran (13.18.%) and Menthyl acétate (12.1%). these results are in disagreement with those reported by other authors for Moroccan peppermint oil composition. Derwich et al. [26] have found Menthone (29.01%) followed by menthol (5.58%) and menthyl acetate (3.34%) as major compound while Debbab et al. [27] have reported that Moroccan peppermint oil was characterized by linalool (60.72 %) and its acetate (20.79 %), as well as geraniol (3.26 %), 1,8-cineol (2.33 %) and limonene (1.54%). Environmental factors such as geography, temperature and genetic factors were considered to play a key role in the chemical composition of essential oils [28, 29].

Table 2: Chemical composition of the essential oil obtained from Mentha piperita L.

	Compounds	RI	% Relative peak area		Compounds	RI	% Relative peak area
Monoterpene hydrocarbons				**16**	Menthol	1171	**46.32**
1	α-thujene	931	0.31	**17**	Terpinen-4-ol	1177	0.04
2	α-Pinene	939	0.32	**18**	α-terpineol	1189	0.03
3	Verbenene	967	0.02	**19**	Carvone	1243	1.02
4	Sabinene	975	1.38	**20**	Neomenthylacetate	1273	0.43
5	β-pinene	979	0.53	**21**	Menthyl acetate	1295	**12.1**
6	α-Phellandrene	1005	0.01	**22**	Isomenthyl acetate	1305	0.82
7	*p*-cymene	1026	0.03	**23**	α-terpinyl acetate	1352	0.03
8	Limonene	1031	3.01	**Sesquiterpene hydrocarbons**			
Oxygenated monoterpenes				**24**	β-Bourbonene	1388	0.37
9	1,8-Cineole	1033	6.06	**25**	α-Gurjunene	1409	0.03
10	Cis-Sabinenehydrate	1070	0.24	**26**	β-Caryophyllene	1418	0.55
11	Linalool	1098	0.05	**27**	α-humulene	1460	0.01
12	Chrysanthenone	1123	0.42	**Monoterpenes hydrocarbons**		**5.61**	
13	Menthone	1152	7.42	**Oxygenated monoterpenes**		**92.95**	
14	Menthofuran	1164	**13.18**	**Sesquiterpenes hydrocarbons**		**0.96**	
15	Neomenthol	1165	4.79	**Total**		**99.52**	

3.2 Experimental design

The observed responses values for different combinations of seven studied variables are listed in Table 3.

Table 3: Experimental design of the hydrodistillation process of *Mentha piperita* L. with the observed answer for each experiment

N° of Experiment	Harvest period	Individuality effect	cutting of leaves	Ratio Material / Water	Extracting time	Heating temperature	Drying	Yield (%)
1	1	1	1	-1	1	-1	-1	1.17
2	1	1	1	-1	1	-1	-1	1.14
3	-1	1	1	1	-1	1	-1	0.98
4	-1	1	1	1	-1	1	-1	0.96
5	-1	-1	1	1	1	-1	1	1.03
6	-1	-1	1	1	1	-1	1	1.02
7	1	-1	-1	1	1	1	-1	1.01
8	1	-1	-1	1	1	1	-1	1.04
9	-1	1	-1	-1	1	1	1	1.1
10	-1	1	-1	-1	1	1	1	1.09
11	1	-1	1	-1	-1	1	1	0.97
12	1	-1	1	-1	-1	1	1	1.01
13	1	1	-1	1	-1	-1	1	1.02
14	1	1	-1	1	-1	-1	1	1.05
15	-1	-1	-1	-1	-1	-1	-1	1.01
16	-1	-1	-1	-1	-1	-1	-1	0.97

3.3 Statistical validation of the postulated model

According to the analysis of variance table (Table 4), we can conclude that the main effect of regression is significant since the risk *p-value* is lower than 0.05. Obviously, the calculation of $F_{Ratio(R/r)}$ (18.62) has shown that it is five times higher than the theoretical value of $F_{(0.05;7,8)}$ at 95% confidence level (3.5). In general, a model has a statistical significance if the calculated F value is at least three to five times greater than the theoretical value [30].

Table 4: Analysis of variance for the fitted model

Source of variance	DF	Sum of squares	Mean squares	$F_{Ratio(R/r)}$	*p-value*
Regression	7	0.0529	0.0076	18.62	0.0002
Residual	8	0.0033	0.0004		
Total	15	0.0562			
R^2	94.2%				

DF: Degrees of freedom; R^2 : Coefficient of determination

The coefficient of determination R^2=94.2% is sufficient. This value gives a good agreement between the experimental and predicted values of the adapted model.

These results are confirmed by those obtained in graph (Figure 1), showing a linear curve for the observed values in term of the predicted ones.

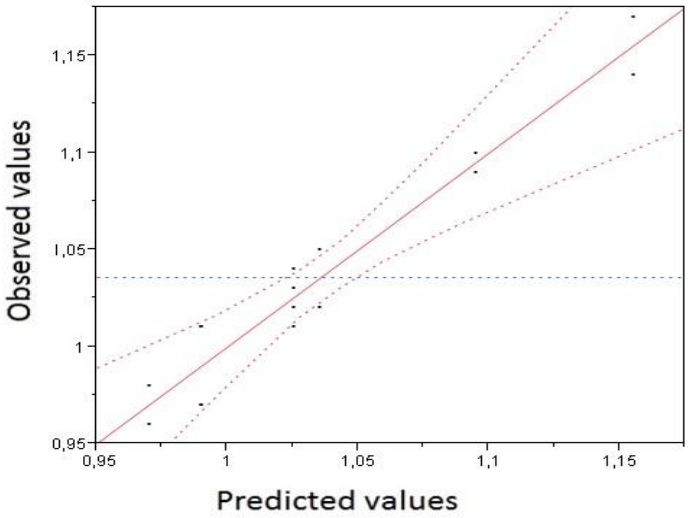

Figure 1: Curve of the observed values in terms of the predicted values

3.4 Study of the factors effects

The main effects of the seven studied variables are shown in table 5. Each coefficient is associated with the values of *t-student* and *p-value*. The values of *t-student* are employed to determine the significance of the regression coefficients and the values of *p* are defined as the lowest level of importance leading to the rejection of the null hypothesis [7]. In general, more the *t-student's* magnitude is larger, more the *p-value* value is smaller, and more the corresponding coefficient term is significant [31].

Table 5: Estimated regression coefficients for the Plackett & Burman design

Name of parameter	Coefficient	Effect	Standard error	*t-student*	*p-value*
Constant	b_0	1.036	0.005	205.53	<0.0001*
Harvest period	b_1	0.017	0.005	3.1	0.0146*
Individuality effect	b_2	0.028	0.005	5.58	0.0005*
Cutting of plan	b_3	-0.001	0.005	-0.12	0.9043
Mass plant/water ratio	b_4	-0.022	0.005	-4.34	0.0025*
Extracting time	b_5	0.039	0.005	7.81	<0.0001*
Heating temperature	b_6	-0.016	0.005	-3.1	0.0146*
Drying	b_7	0.001	0.005	0.12	0.9043

*: Statistically significant factor for a level of confidence of 95%.

The results show that only the factors b_3 and b_7, which are related to the plant's cutting and drying respectively, doesn't have any influence on the hydrodistillation process since their signification risk is superior than 0.05. These results are confirmed by the two graphs in Figure 2. Indeed, the graph (Figure 2a) shows that all factors have a statistically significant effect except the leave's drying and cutting which showed a negligible statistical effect. The graph (Figure 2b) shows that the extracting time is the more influential factor on *Mentha pepperita* L. essential oil extraction followed respectively by the individuality effect, the material/water ratio, the harvest period, the heating temperature. The value of the constant b_0 is equal to 1.03.

3.5 Fitted Model

The statistical mathematical model representing the response in terms of the most influential variables is:

$$Y = 1.036 + 0.017\,X_1 + 0.028\,X_2 - 0.022\,X_4 + 0.039\,X_5 - 0.016\,X_6 + \varepsilon$$

The calculated experimental error (ε) from the residual mean square was 0.023.

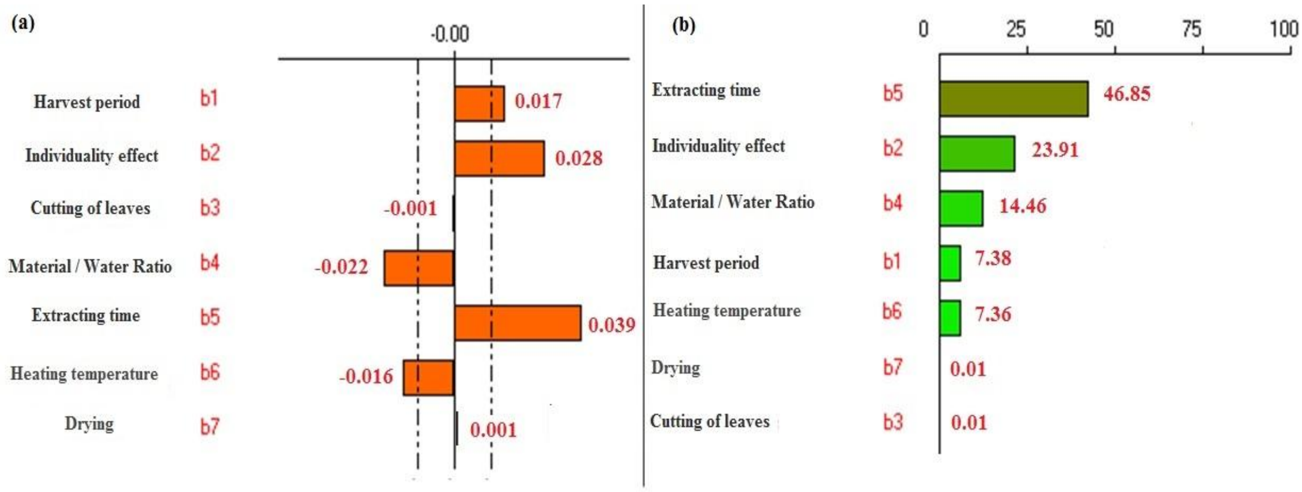

Figure 2: (a): The factors effect's graph; (b): the contribution percentage of each factor to the studied response

3.6 Statistically negligible parameters

Figure 3 shows that there is a small increase in essential oil yield between dried and fresh plants and between the cut and entire leaves, but the test on coefficients b_3 and b_7 (table 5) shows that these two factors don't have any influence on the hydrodistillation operation, since there signification risk *p-value* is superior to 0.05 (0.9 for both coefficients).

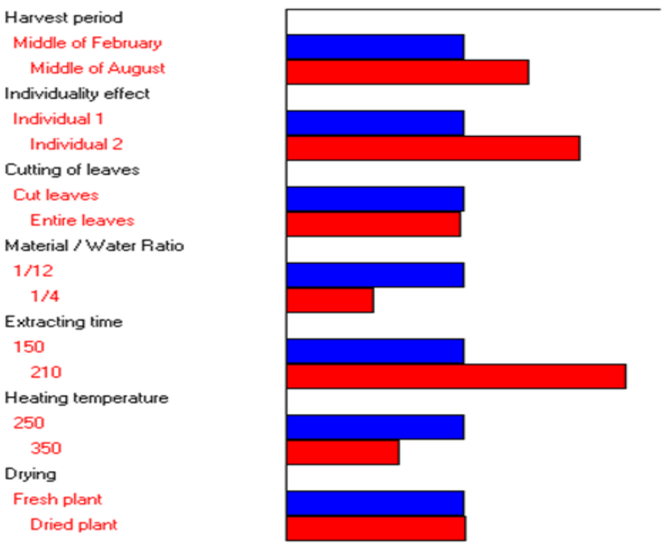

Figure 3: Variation of the response (Essential oil yield) in terms of the variation of each parameter

3.6.1 Cutting of leaves

Theoretically, the leave's cutting must have a large influence on the yield because it induced the occurrence of an important surface contact between the plant material and water, and then facilitating the essential oil extraction process. However, the results obtained (table 5) revealed a negligible effect of cutting. This can be explained by the fact that the sites of oil biosynthesis in peppermint are localized in the secretory cells of the glandular trichomes [32], these latter are located upon the aerial surfaces [33].

3.6.2 Drying

A small yield increase was observed between fresh and dried plants (figure 3). This result is similar to those found for other plants such as *Rosmarinus officinalis* L. [34] and *Tetraclinis articulata* [35] which shows that plant's drying for one week leads to remarkable increase in yield. However, in our case this increase was not statistically significant.

3.7 Statistically significant parameters

3.7.1 Extracting time

The two graphics (Figure 2) show that extracting time (factor b_5) is the most influential factor on the hydrodistillation operation with a coefficient of 0.039. This factor contributed by 46.85% to the variability of the studied response. Obviously, the time influences directly the hydrodistillation operation and its impact on such operation has been demonstrated by several authors [7-11, 21].

3.7.2 Individuality effect

The individuality effect is the second factor affecting the hydrodistillation process with a coefficient of 0.028 and 23.91% of contribution in the yield variability. The results show that the passage from the individual 1 to individual 2 generates an increase in the essential oil yield. This difference in essential oil yield from one individual to another can be explained by the development stage of the plant organ (leaf, flower and fruit ontogeny), or by genetic and environmental factors [36].

3.7.3 Vegetable material / Water Ratio

The third factor showing a significant influence on the yield is the ratio between vegetable material and water. This factor has a coefficient of –0.022 and a contribution of 14.46%. The minus sign indicates that the passage from the minimal level (the ratio 1/12) to the maximal level (the ratio 1/4) causes a decrease in the essential oil yield. Several studies have reported that the increase in the ratio of vegetable material and water produce a decrease in yield [7, 8, 10]. This decrease can be explained by the fact that the existence of high amount of plant material in water prevents the water vapor formed in the bottom of the flask to rise up in the condensation tube which induces a yield reduction [37].

3.7.4 Harvest period

The harvest period comes in the fourth position with a coefficient of 0.017 and a contribution of 7.38%. This result indicates that yield becomes more important in the middle of August compared to the middle of February. Our results are in accordance with Hussain's ones [38] who have demonstrated that essential peppermint oil yield is higher in summer (when the plants were in full bloom) than in winter (when the plants reached the end of their growing cycle). Furthermore, William et al. [39] has reported that August is the best harvest time for commercial production of the essential oil.

3.7.5 Heating temperature

The last factor having a significant effect on the yield is the heating temperature, with a coefficient of -0.016 and a contribution of 7.36%. The increase of heating temperature enhances the steam condensation's flow and then, reduces the essential oil yield. Indeed, a big increase of the steam condensation's flow leads to a decrease of the condensate's residence time in the decanter, which does not leash time for essential oils to be separated from the liquid [40]. These results are concordant with those found by other authors [7, 8, 37].

Conclusion

In the end of this study, we were able to evaluate and classify the effect of operating conditions on the essential oil yield of *Mentha piperita* L. by using the experimental designs methodology. The results show clearly that this technique is an appropriate strategy for the screening of the factors affecting the hydrodistillation of peppermint. The Placket and Burman design lead to a first-order polynomial model and the statistically significant coefficients are related to the most influential factors on the response. The statistical validation of the obtained model has allowed us to confirm that all the studied factors have a significant effect on the hydrodistillation of *Mentha piperita* L. leaves except the leave's drying and cutting which showed a negligible statistical effect.

To complete this study, an optimization study must be conducted. It will search for the optimal operating conditions to have a better essential oil yield, and this, through the use of the response surface methodology which is more advocated for this type of study. The optimization study will concern only the continuous operating factors such as time processing, mass plant/water ratio, and heating temperature.

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