Optimization of Walnut Nut Extraction Yield by using the Response Surface Methodology

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Abstract: ***This study focuses on improving the aqueous extraction of walnut brown. Optimal extraction conditions, i.e. pH, temperature and duration, were determined using an orthogonal Composite Centred Design (CCD) with iso-rotation. The ANOVA results indicated that pH has a linear and quadratic effect, temperature, and interaction terms (Duration-pH), (T°-Duration), have a significant influence on extraction yield. In addition, the proposed second-order regression model is consistent with the experimental data with a high coefficient of determination (R- square (adjusted) = 96.53%). According to the response optimization of Statgraphycs software, the optimum conditions for extraction of walnut brown are: pH 8, temperature 80 °C and extraction time 9 minutes. Under these conditions, the experimental extraction yield is 96%, which is close to the value predicted by the statistical design (102%).***

Keywords: Modelization, Extraction, Walnut, Experimental Design, Optimization

1. Introduction

A chemical called "juglone" (5-hydroxy-1, 4-napthoquinone) is naturally present in all walnut components. Walnut brown and walnut leaves are rich mainly in C.I. juglone. 75500 (I.C. Natural Brown 7). This substance is used in textile dyeing, polyamide wool, cotton and linen as well as in cosmetics to dye hair. It is also a powerful modifier of the anatomical elements of the skin [1]. It has a mortifying effect on the diseased epidermis and regenerates it. Leaves and brown would therefore be particularly useful in certain dermatoses: eczema, psoriasis, dandruff.

On the other hand, its antimicrobial properties [2] would be useful for treating acne due to Propionibacteium acnes.

In this work we study the aqueous extraction of walnut brown. The effects of pH (1.6-13.8), temperature (26.4-93.64 degrees Celsius), and duration (4.77-55.23 minutes) on Juglone extraction were studied by the experimental design methodology with a three-factor, five-level composite centred design (CCD). UV-visible spectroscopy showed a clear 30% increase in yield for pH variation from 4 to 11. The analysis of the experimental design (figure on effects) allowed us to note that an increase in the extraction time from 15 minutes to 45 minutes reduces the yield of the dye extraction by 4% while the increase in temperature improves it n by 16%. The size of the kernels that make up the walnut kernel's crushing influences the extraction yield. We used a grinding mill with a grain size of less than 2mm [3]. In order to model and optimize extraction efficiency according to parameters, pH, temperature and duration, we used a central composite designe (CCD) [4]. The plotting of the iso-responses and curves from the equation of the validated model allowed us to obtain the optimal conditions of pH (8), temperature (80°C) and extraction time (9mn) for a maximum yield of 96%. This water extraction process is environmentally friendly and economically very cost-effective.

2. Experimental Protocol

2.1. Preparation of walnut nut

The walnut nut (Figure 1) becomes black by drying and naturally detaches from the nuts. In Morocco, these hulls are sold dry. Extraction of brown pigment does not require expensive prior preparation. Dry, grind and sift to homogenize the grain size.

Figure 1 : Nuts after drying

Grinding increases the exchange surface between the grinding and the extraction solvent and facilitates the extraction of the coloring matter, hence the advantage of reducing the particle size to a smaller diameter. Dried roots placed in a spice mill and ground into powder and stored in glass jars at room temperature (25-27°C). This grinding was subjected to a grain size analysis by passing it through electromagnetic sieves -Tamizadora electromagnetica (TZBA- 200N).

Figure 2: Electromagnetic Screen (CISA BA- 200N)

Based on the resulting particle size curve, it is noted that 80% of the aggregate is smaller than 2 mm. This ensures a large surface of contact with the solvent and consequently a good extraction efficiency

Figure 3: Nut brown powder

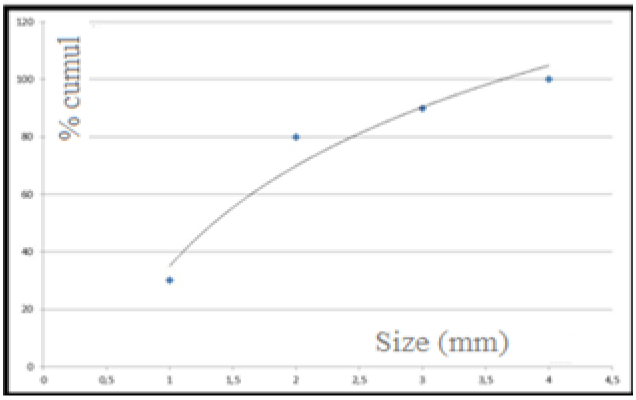

Figure 4: Particle size of walnut-nut broyat

2.2. Methodology of design experimental and surface response (RSM)

The parameters qualitatively influencing the extraction yield, selected from the results of the screening design [3], are studied in this part using a composite centred design, in which the response is the extraction yield of walnut (Yield% = Final Abs/ Initial Abs), with Initial Abs = 1.95 which corresponds to the absorbance of the dark brown solution of the brown or walnuts (color index 7 of natural brown), and variables X_1, X_2, and X_3 respectively, are pH, temperature, and extraction time. The values of the transformed or coded variables Xj and the values of the actual variables are summarized in Table 1. The response surface methodology (RSM) is the second part of the experimental design method. This technique is intended to quantitatively determine changes in the response function of significant influences

Table 1: Values of variables at 5 levels, used in the centred composite design

Actual variables (x_j)	Coded Variables X_1, X_2, X_3				
	-1,682	-1	0	1	1,682
pH	1,613	4	7,5	11	13,81
Temperature (°C)	26,4	40	60	80	93,64
Duration (mn)	4,77	15	30	45	55,23

2.3 Experimental results

Table 2 lists the coded values of the three important factors as well as the value of the experimental response of the extraction yield and the value estimated by the model. From these results, it appears that pH and temperature have a strong impact on extraction. Therefore, the yield ranges from 10.62% to 55.538% for a pH variation of 1.61 to 13.83, for a temperature of 60°C and a extraction time of 30 minutes. It can also be noted that the extraction yield of walnut for a temperature variation of 26.4 °C to 93.64°C, increases from 51.282% to 80.923% for a duration of 30 minutes and at pH = 7. While it drops from 73.33% to 64% for extraction time ranging from 4.77 minutes to 55.23 minutes.

2.3.1. Data analysis and model equation

The ANOVA variance analysis was conducted in a 95% confidence interval, implying that factors in the p <0.05 value have a significant impact on the extraction yield of walnut brown. The values obtained by this analysis (Table 3), show 5 effects that have p values less than 0.05, indicating that they are significant at the 95.0% confidence level. The R-square statistic indicates that the model accounts for 98.57% of the variability in Yield. The adjusted R-square statistic, which is more suitable for comparing models with different numbers of independent variables, is 96.53%. The adjusted R^2 and R^2 coefficient of determination are close to 1, confirming the correlation between the experimental and model predicted values. The fact that these two coefficients are close to each other also indicates that the model does not include the non-significant variables. The standard error of the estimate shows that the standard deviation of residues is 0.073. The average absolute error (MAE) of 0.039 is the average residue value. Thus, the results analyzed by ANOVA show linear and quadratic effects of pH and a linear effect of temperature on yield.

The model predictive equation for Yield is given below:

The equation of the adjusted model is:

$$\text{Yield\%} = 72.98 + 13.70X_1 + 7.48X_2 - 13.55X_1^2 + 6.45X_1X_3 - 14.02X_2X_3 \quad (1)$$

The significant positive effect of pH on extraction is noted. Temperature also influences positively. However, the duration has a slight negative effect and remains non-significant. It can be clearly seen that pH has a large influence on yield with a linear and quadratic effect. The term X_1 and X_3 has a positive influence, indicating that increasing the pH and the duration of extraction at the same time improves Yield. The term X_2X_3 interactions, on the other hand, has a large negative impact on yield, meaning that increasing temperature and sustaining extraction tends to significantly reduce yield. Our results have been confirmed by the Pareto diagram (Figure 5). This extraction yield diagram shows that pH and temperature are the most significant factors.

In Table 2, we also note that the majority of the experimental values and values predicted by the quadratic model equation are very close to each other, confirming the validity of the model

Table 2: Experimental and model-estimated values

Exp	X_1	X_2	X_3	Y_{exp}%	Y_{estim}%
1	-1	-1	-1.	47,69	50,04
2	0	0	0	72,62	72,98
3	-1	-1	1	46,31	44,43
4	-1	1	1	32,77	33
5	1	1	1	73,85	71,64
6	1	1	-1	89,85	89,51
7	1	-1	-1	50,62	48,17
8	-1,682	0	0	10,62	9,54
9	-1	1	-1	75,03	76,68
10	1	-1	1	88,26	86,39
11	1,682	0	0	57,54	59,73
12	0	-1,682	0	53,28	55,08
13	0	1,682	0	80,92	80,24
14	0	0	-1,682	73,33	72,93
15	0	0	1,682	64,82	65,34
16	0	0	0	72,72	72,98
17	0	0	0	72,62	72,98
18	0	0	0	72,62	72,98

F_{th}= $F_{(0,05)}$(1; 10) = 4,95 * : F_{exp}>;4,95
R-Squared = 98,57 %
R- Square (ajusted) = 96,53 %

Table 3: Analysis of the Variance in the Absorption of Nuts

Source of variation	Sum of squares	Degree of freedom	Mean square	F_{exp}	P-Value	Signifiance
X_1	1776,42	1	1776,42	126,66	0,0000	*
X_2	602,919	1	602,919	42,99	0,0003	*
X_3	20,0682	1	20,0682	1,43	0,2706	No
X_1^2	2147,09	1	2147,09	153,09	0,0000	*
X_1X_2	3,80103	1	3,80103	0,27	0,6187	No
$X_1 X_3$	228,572	1	228,572	16,30	0,0050	*
X_2^2	17,6268	1	17,6268	1,26	0,2992	No
X_2X_3	1080,53	1	1080,53	77,05	0,0001	*
X_3^2	0,13622	1	0,13622	0,01	0,9243	No
Err total	53,1254	1	53,1254	3,79	0,0927	
Tot.corr	98,1722	7	14,0246			

F_{exp} : Fisher –Snedecor Experimental F_{th}= $F_{(0,05)}$(1; 10) = 4,95 * Significant to 95% : F_{exp}>;4,95 **;**
Critical Fisher-Snedecor according to the table.
R-Squared = 98,57 %; R- Square (ajusted) = 96,53 %

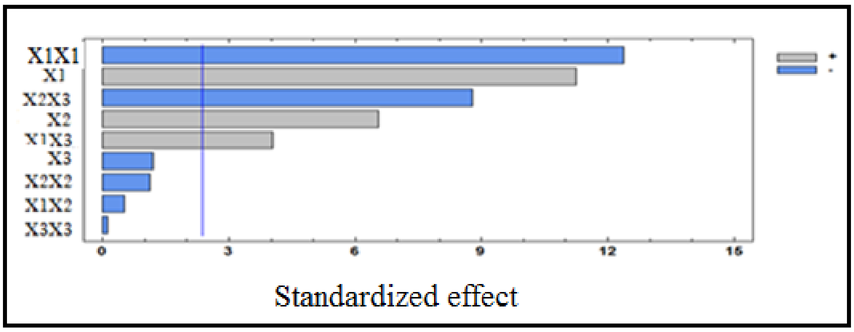

Figure 5: Pareto diagram

2.3.2. Effects Graph

The effect graphs (Figure 6) visualize the evolution of Yield in relation to each of the operating parameters studied. The significant positive effect of pH on extraction is noted, which is consistent with the model equation. Temperature also influences positively. However, duration has a slight negative effect. Based on this analysis, and in particular the sign of the effect of the extraction time. The effects graph also visualizes that beyond the neutral pH (coded value = 0). This graph only discusses the individual effect of each factor on Yield. However, it allows us to select the duration of the extraction as a factor to set at a minimum value in order to achieve maximum efficiency while optimizing the extraction time.

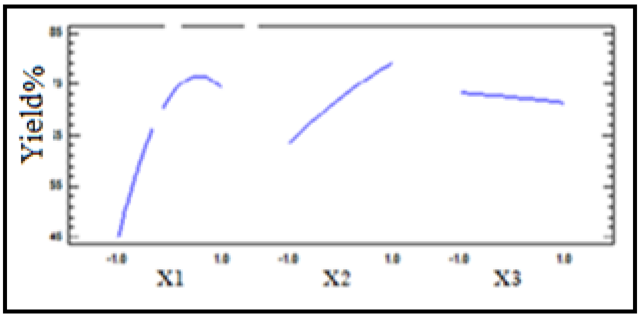

Figure 6: Graph of Effect of Factors on yield

2.3.2 Iso-response Contours and optimized response

We visualize in the figure below the variability of the yield according to two variables while the third is kept fixed. We set the X3 extraction time value to -1.4, which corresponds to a duration of 9 minutes. The study of contours graphically visualizes the variability and optimization of Yield. Each curve represents an infinite number of combinations between two variables, and the third variable is kept at a constant level. Optimal values leading to a 102% yield are checked in our case and they appear on the graph of the iso-response curves. The corresponding actual values are: X1 (pH) = 8; X2(T°C) = 80°C; and X3 (Duration) = 9 mn. The following table 4 summarizes the optimal values of the three parameters studied.

Experimental validation of these optimum conditions was carried out, which allowed us to obtain a real return of 96%. The second-order forecast model tested by the analysis method (ANOVA) is validated. This analysis showed that the results of the model are highly significant and in good alignment with the experimental results.

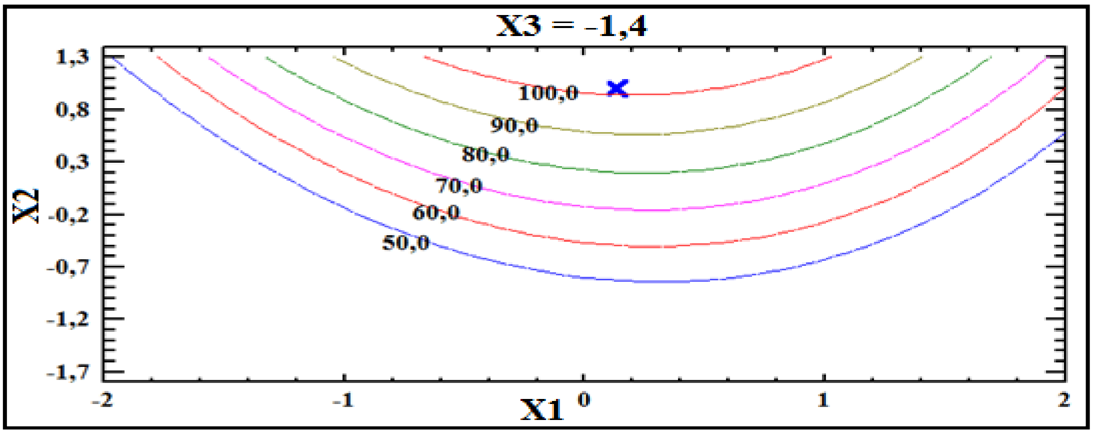

Figure 7: Iso-response Contours of walnut brown extract

Table 4: Low, high, and optimal levels of all three parameters

Factor	Low	Up	Optimum
X_1	-1,682	1,682	0,134
X_2	-1,682	1,0	1,0
X_3	-1,682	1,0	-1,4

2.2 Spectroscopic characterization

The infrared absorption spectrum of the samples was recorded using a PERKIN ELMER FTIR spectrometer scanning the wavelength range 4000-450 cm-1 (Figure 8). The sampling technique was used by grinding approximately 1mg of the product into 100 mg of KBr. highlight the main functional groups that are part of the walnut brown chromophore groups.

These functional groups of coloring extracted from the walnut brown analyzed, show a strong band in the wavelength range 3300-3500 cm-1, indicating the presence of OH and CH groups, and thus alcohols, phenols and alkanes. The aldehyde groups, C = O, are identified by stretching in the range 1600-1800 cm-1. In the 1600-1500 cm-1 range, the presence of aromatic and saturated C-C structures is present in naphtoquinone as naphthalene derivatives. The C-O groups were identified in the range 1100-1000 cm-1. These findings are consistent with other discoveries in the literature, [5-6]

To highlight structural changes in the natural dye as a function of pH, we used UV/VIS spectroscopy. UV spectra were performed using a device (Jenway 6715), 20g of the finely crushed brown is added to 100mL of distilled water. A sample of the mixture is placed in the UV cell and then scanned between 200 and 1000 nm.

The spectrum resulting from this analysis is shown in Figure 9. Two peaks appear around the 230 and 290 nm wavelength regions, respectively, with a narrow band in the 400 nm region, which is characteristic of the juglone spectra [7]

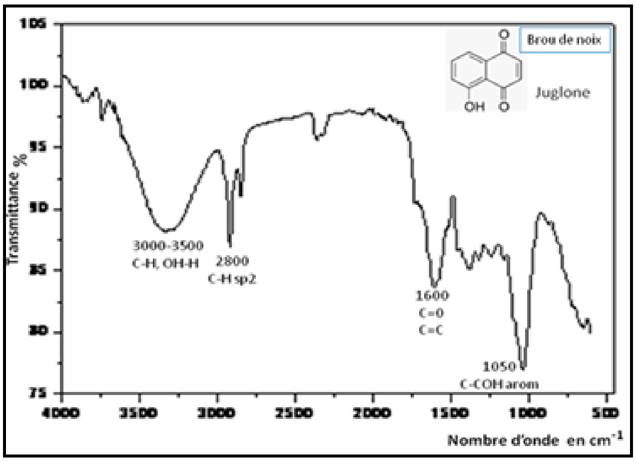

Figure 8: FTIR spectrum of walnut brown

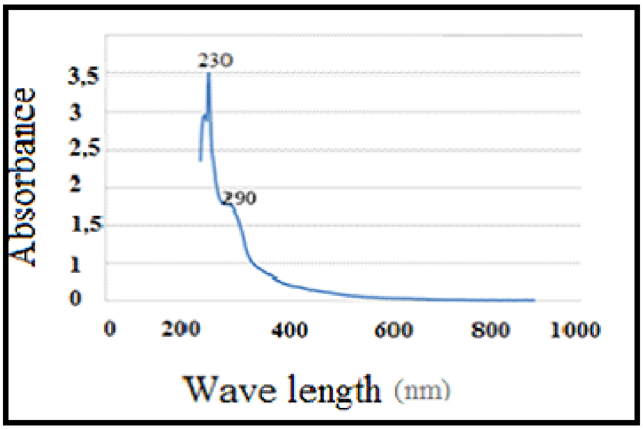

Figure 9: UV Spectrum of walnut nut

3. Conclusion

The results obtained have also led to the identification of new avenues of research, namely the aqueous extraction of the natural brown IC 7 dye, to increase its effectiveness. Such a field was not well mastered in the ancestral culture of extraction of natural dyes. The result was to model and optimize Yield based on pH, temperature and extraction time. The good agreement between theoretical and experimental values further encourages its use.

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