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Optimization of Oil Extraction from *Nigella sativa* Seeds by Pressing Using Response Surface Methodology

Naima Hadjadj, Hakima Acheheb, Feriel Sabrine Aitchaouche, Djamila Belhachat and Ali Ferradji
Department of Food Sciences, High National School of Agronomy Algiers, Algeria

Corresponding Author: Ali Ferradji, Department of Food Sciences, High National School of Agronomy Algiers, Algeria

ABSTRACT
The oil of *Nigella sativa* seeds was extracted using hydraulic press. The pressure, temperature and thickness of cake have been optimized, using the Response Surface Methodology to obtain a high oil yield. The physicochemical properties of the seeds and of its oil extracted were also studied. Results showed that the oil *Nigella sativa* seeds is rich in unsaturated fatty acids (81.25%) and saturated fatty acids (18.74%). The unsaturated fatty acids identified, using gas chromatography, are linoleic acid (54.97%), oleic acid (24.13%) and eicosenoic acid (3.23%). The saturated fatty acids are palmitic acid (12.50%), behenic acid (2.623%) and arachidic acid (0.338%). The cross section of the seed showed that the oil is localized in the cotyledons and integuments. Statistical analysis revealed that data were adequately fitted in second-order polynomial model. The linear and quadratic terms of temperature, pressure and thickness of cake had significant effects on the oil yield ($p<0.05$). The optimal conditions to obtain the maximum oil yield (22%) were found to be 120 bars, 60°C and 1.30±0.047 cm for thickness cake.

Key words: Fatty acids, *Nigella sativa* oil, response surface methodology, hydraulic press, oil extraction

INTRODUCTION
Nigella sativa is belongs to the family of Ranunculaceae, it grows in many areas in Algeria. The *Nigella sativa* seeds are commonly called sanoudj in Algeria. Algerian people use these seeds as seasoning for foodstuffs like bread and traditional cake. There are several reports about the beneficial effects of the *Nigella sativa* seeds and their extracts on the human health. Indeed the seed oil is known for its medicinal proprieties as an antidiabetic, antihistaminic, antihypertensive, anti-inflammatory, antimicrobial, antitumor, antioxidant and anti- eicosanoid (Houghton *et al*., 1995; Riaz *et al*., 1996; Ali and Blunden, 2003). There are also many works related to the study of volatiles oils and the physicochemical characteristics of fixed oil (Atta, 2003; Ramadan and Morsel, 2003; Cheikh-Rouhou *et al*., 2007; Kaskoos, 2011). However, there is no available informations about the optimization of oil extraction by pressing. The purpose of this work is to report the general characteristics of *Nigella sativa* seeds and its oil and the optimization of oil extraction by pressing using the response surface methodology.

MATERIALS AND METHODS
Materials: The *Nigella sativa* seeds were purchased from local market. The seeds have the following characteristics: Small, black, flat, funnel-shaped, 0.2 cm long and 0.1 cm wide.

Analytical methods: All analytical determinations were performed in triplicate for each sample with the standard deviation.

Sample preparation for oil extraction by hydraulic press: Oil extraction is carried out from *Nigella sativa* seeds using hydraulic press described by Acheheb *et al.* (2012). The ground sample, having a particle size less than 1 mm, is compressed, during 60 min, at various pressures (50, 90 and 120 bars), temperatures (25, 40 and 60°C) and thickness cake (1.30±0.047, 2.80±0.190 and 4.04±0.28 cm). The oil yield is the ratio between the mass of the oil extracted and mass of the sample (g 100 g^{-1} dry weight).

Determination of chemical and botanical properties of *Nigella sativa* seeds: The moisture content was determined using Infra-humid meter (Sartorius). Crude proteins, lipids and ash were determined by the method described by AFNOR (2000). Carbohydrates were evaluated by difference using the following equation:

100-(weight in gram (proteins+fat+water+ash) in 100 g of food

(FAO, 1998). The observation of transverse section of *Nigella sativa* seed was carried out using the photonic microscope.

Determination of physicochemical characteristics of *Nigella sativa* seeds oil

Oil content: Oil is extracted from 10 g of ground seeds in a Soxhlet extractor using hexane as a solvent. The result is expressed as the percentage of lipids in the dry matter of seeds.

Determination of fatty acids composition: The methyl esters were prepared according to AFNOR (2000) and analysed using a Thermo Finnigan Gas Chromatograph equipped with flame ionisation detector. The separation of fatty acid methyl ester was carried out at 190°C on a 30×0.32 mm capillary packed with the polar stationary phase Nona Fluoro Pentanoic Acid (NFPA), with nitrogen as a carrier gas. The temperature of detector was 230°C and the injection block was recorded as 210°C. Peaks were identified by comparing the retention times with those of a mixture of standard methyl esters. Peroxidizability Index (PI) was evaluated using the equation of Song *et al.* (2000):

$$PI = (\%\ monoenoic \times 0.025)+(\%\ dienoic \times 1)+(\%\ trienoic \times 2)+(\%\ tetraenoic \times 4)+ (\%\ pentaneoic \times 6)+(\%\ exaneoic \times 8) \quad (1)$$

Experimental design: Response surface methodology was used to optimize the oil extraction from *Nigella sativa* seeds by hydraulic press. The effects of independent variables, temperature (25-60°C), pressure (50-120 bars) and thickness cake (1.30-4.04 cm) on oil extraction were studied using the Box-Behnken design. The coded and uncoded levels of different process variables are indicated in Table 1.

The second response surface model used to fit the experimental data has the following form:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta ii x_i^2 + \sum_{i=1}^{n} + \sum_{i=1}^{n-1} \times \sum_{J=i+1}^{n} \beta_{ij} x_i x_j \quad (2)$$

where, Y is the response (oil yield in%), β_0, β_i, β_{ii} and β_{ij} are constant coefficients of intercept, linear, quadratic and interaction terms, respectively xi and xj are coded independent variables (Montgomery, 2005; Bradley, 2007). Analysis was conducted using Design-Expert® v. 8.3.1. The quality of the fitted model was evaluated by the analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Botanical characteristics of *Nigella sativa* seeds: Figure 1 shows a transverse section of *Nigella sativa* seed. The seed is covered externally by an outer integument. The outer integument is followed by 2-4 layers of parenchymatous cells with a thick wall and by a pigmented layer (inner integuments) filled with brown compounds (oil). Below the inner integument, there is a cuticle followed by an endosperm consisting of rectangular to polygonal cells with thick wall filled with globules oil. These observations agree with those reported by Esau (1965). The layer of globules oil of the seed coat is separated from the endosperm by a cuticle. This observation allows concluding that oil of *Nigella sativa* seed is located in the cotyledons and integuments.

Table 1: Coded and uncoded levels of different process variables used in Box-Behnken design

	Symbol		Levels	
Independent variables	Coded	Un-coded	Coded	Un-coded
Temperature (A, °C)	A	T	-1	25
			0	40
			+1	60
Pressure (B, bars)	B	P	-1	50
			0	90
			+1	120
Thickness cake (C, cm)	C	TC	-1	1.30
			0	2.80
			+1	4.04

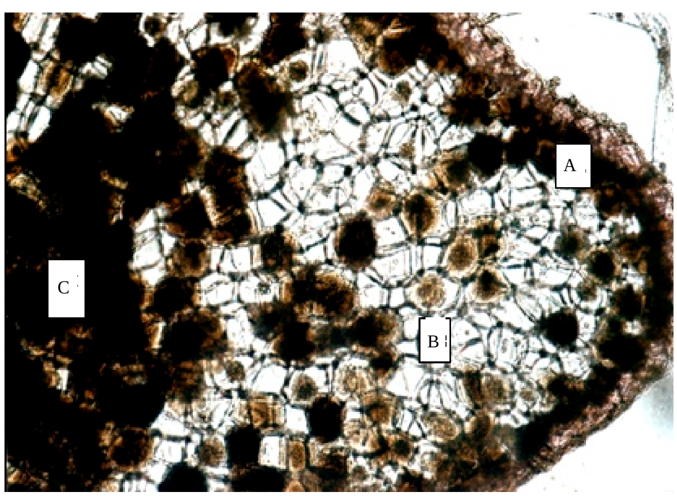

Fig. 1: Transverse section of *Nigella sativa* seed, A: Oil of seed coat, B: Endosperm and C: Globules oil

Table 2: Physicochemical properties of *Nigella sativa* seeds (g 100 g^{-1} dry weight)

Constituants	Value
Moisture	9.61±0.14
Crude protein	20.69±0.84
Ash	4.04±0.29
Carbohydrate (by difference)	25.29
Crude fat	40.37±1.70

Table 3: Fatty-acids composition of the oil *Nigella sativa* seeds (Unit: relative peak area%)

Fatty acids	Percentage	Retention time (min)
Palmitic acid (C16:0)	12.538	8.74
Palmitoleic acid (C16:1)	0.250	9.18
Stearic acid (C18:0)	3.239	12.50
Oleic acid (C18:1)	24.135	13.17
Linoleic acid (C18:2)	54.970	14.74
Linolenic acid (C18:3)	0.181	17.07
Arachidic acid (C20:0)	0.338	19.56
Eicosenoic acid (C20:1)	1.722	20.66
Behenic acid (C22:0)	2.623	23.56
Σ SFA	18.740	
Σ UFA	81.259	
Σ MUFA	26.107	
Σ PUFA	55.151	
Σ UFA/Σ SFA	4.336	

Chemical proprieties of *Nigella sativa* seeds: Table 2 shows the chemical composition of *Nigella Sativa* seeds. Moisture content of whole seeds was 9.61±0.14 (g 100 g^{-1} on dry weight). Oil contents were 40.37±1.70 g 100 g^{-1} on dry weight, this reflects the importance of using such seeds for oil production. Crude protein level was 20.69±0.84 g 100 g^{-1} on dry weight, show that the *Nigella sativa* seeds have an important nutritional value. The other components were in the following decreased order, Carbohydrates total 25.29 g 100 g^{-1} on dry weight and ash content 4.04±0.29 g 100 g^{-1} on dry weight. The carbohydrate content indicates that *Nigella sativa* seeds can be a source of energy.

Fatty acids: The fatty acids composition of oil *Nigella sativa* seeds and their percentages are presented, in order of their elution in the column, in Table 3. Palmitic, stearic and behenic acids were the major satured acids, whereas linoleic and oleic were the major unsatured acids. The content of linoleic acid was much higher than that of oleic acid. The presence of fairly high amounts of the essential fatty acid linoleic, suggests that the *Nigella sativa* seeds oil is highly nutritious. Linoleic acid is also a precursor of arachidonic acid, another essential fatty acid (Ali *et al.*, 2012). However, (Cater and Denke, 2001) have reported that behenic acid, despite its low bioavailability, is considered as a factor in the increase of cholesterol in humans.

The results of this study revealed also that the oil *Nigella sativa* seeds contains 1.722% of eicosenoic acid (gadoleic acid, C20:1) but not eicosadienoic acid (C20:2). These results are in accordance with those of Ustun *et al.* (1990). On the other hand, Aitzetmuller *et al.* (1997) and Matthaus and Ozcan (2011) have reported that these fatty acids (C20:1 and C20:2) could be considered as chematoxonomic characteristics of *Nigella* and as an identity criterium of *Nigella sativa* seeds oil. The values of peroxidizability index and of unsaturated/saturated ratio which are, respectively 55.45% and 4.33, show that the crude oil extracted is stable to auto-oxidation rancidity during storage.

Response surface analysis: The seventeen generated experiments with the values of various responses to different experimental combination for coded variables are given in Table 4. A large variation in the oil yield, from 3.81-22.64 g 100 g^{-1} on dry weight was observed for different experimental combinations. The experiments were conducted in accordance with the Box-Behnken design to find the optimal combination of pressure, temperature and thickness of cake for maximum oil yield.

The results of analysis of variance carried out to estimate the quality of the fitted second order response surface model are shown in Table 5. The sign and magnitude of the coefficients allows interpreting the effect of the variable on the response. The negative sign of coefficient indicates that when the level of the variable increases the response decreases while the positive sign indicates an increase in the response. The model F-value of 61.36 reveals that the model is significant. Values of "Prob>F" less than 0.0500 indicate that model terms are significant. In this study A, B, C, AB, A^2, C^2 are significant model terms.The high coefficient of determination (R^2) which is of 0.987 shows that the fit of the model is good. The value 8.88% of coefficient of variance (CV) indicates that the deviations between experimental and predicted values are low. Figure 2 confirms the good agreement between the experimental and predicted values for oil yield from *Nigella sativa* seeds. In conclusion the final model for the response variable oil yield is as following:

$$\text{Oil yield(g 100 g}^{-1}\text{ on dry weight)} = +8.29+7.04\times A+1.91\times B-0.88\times C+1.38\times A\times B+0.21\times A\times C+1.05\times B\times C+2.77\times A^2+0.79\times B^2+1.36\times C^2 \quad (3)$$

Response surfaces and contour plots: Results of analysis of variance have shown that the linear term of temperature (A), pressure (B) and thickness cake (C) had a significant effect on the oil extraction yield (Table 5). The effects of linear terms are followed by those of quadratic term of temperature (A^2), thickness cake (C^2) and interaction between temperature and pressure (AB).

Table 4: Experimental conditions and observed response values of BBD

Run	Coded A	Coded B	Coded C	Un-coded T	Un-coded P	Un-coded TC	Response of oil yield (g 100 g^{-1} dry weight)
1	-1	-1	0	25	50	2.80	3.81
2	1	-1	0	60	50	2.80	16.58
3	-1	1	0	25	120	2.80	4.37
4	1	1	0	60	120	2.80	22.64
5	-1	0	-1	25	90	1.30	7.63
6	1	0	-1	60	90	1.30	19.84
7	-1	0	1	25	90	4.04	4.58
8	1	0	1	60	90	4.04	17.64
9	0	-1	-1	40	50	1.30	9.77
10	0	1	-1	40	120	1.30	12.00
11	0	-1	1	40	50	4.04	6.78
12	0	1	1	40	120	4.04	13.23
13	0	0	0	40	90	2.80	8.29
14	0	0	0	40	90	2.80	8.29
15	0	0	0	40	90	2.80	8.29
16	0	0	0	40	90	2.80	8.29
17	0	0	0	40	90	2.80	8.29

T (A): Temperature, P (B): Pressure, TC (C): Thickness cake

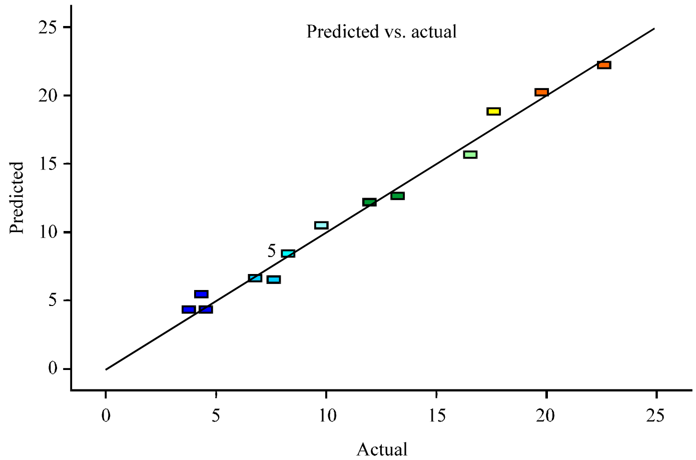

Fig. 2: Predicted vs. observed values for extraction yield of oil from *Nigella sativa* seed

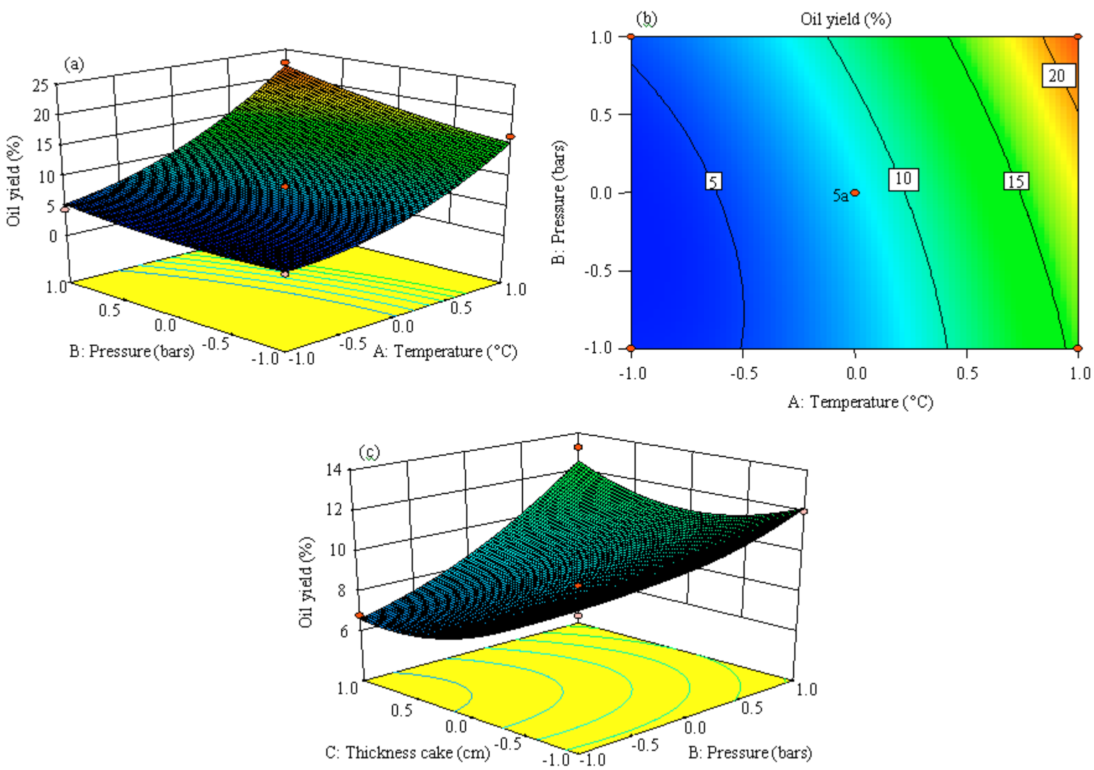

Fig. 3(a-c): Surface plot and contour of oil yield from *Nigella sativa* seeds as a function of (a) Temperature and pressure, (b) Contour plot of temperature and pressure and (c) Thickness cake and pressure

The effect of independent variables (temperature, pressure, cake thickness) on the dependent variable (oil yield) is indicated by the response surfaces plots developed from Eq. 3 (Fig. 3a-c).

Table 5: Analysis of variance (ANOVA) for the response surface quadratic model for oil yield from *Nigella sativa* seeds using hydraulic press

Source	Coefficients	Sum of squares	df	Mean of squares	F-value	Prob>F
Model	8.29	490.2600	9	54.470	61.36	<0.0001
A- Temperature	7.04	396.3500	1	396.350	446.44	<0.0001
B- Pressure	1.91	29.2600	1	29.260	32.96	0.0007
C-Thikness cake	-0.88	6.1400	1	6.140	6.92	0.0339
AB	1.38	7.5600	1	7.560	8.52	0.0224
AC	0.21	0.1800	1	0.180	0.20	0.6656
BC	1.05	4.4500	1	4.450	5.01	0.0601
A^2	2.77	32.2800	1	32.280	36.36	0.0005
B^2	0.79	2.6400	1	2.640	2.97	0.1285
C^2	1.36	7.8300	1	7.830	8.82	0.0208
Residual		6.2100	7	0.890		
Lack of fit		6.2100	3	2.070		
Pure error		0.0000	4	0.000		
R^2		0.9875				
Adj R^2		0.9714				
Pred-R^2		0.7997				
SD		0.9400				
CV%		8.8800				
Press		99.4300				

Figure 3a shows that the oil yield increased with increase in temperature and in pressure. Figure 3c shows that the oil yields decrease with increase of thickness cake. The contour plots can spot the optimum response approximately. The interactions of temperature-pressure have positive effect on oil yield. The optimum value response (>20%) was observed for the high level of independent variables and visualized in Fig. 3b at the corner of the top and right side.

CONCLUSION

This study has shown that the Response Surface Methodology is a effective way to determine the optimal conditions for oil extraction from *Nigella Sativa* seeds by pressing. Analysis of variance has shown that the effects of all the process variables including temperature, pressure and thickness cake were statistically significant. Polynomial model was obtained for predicting oil yield. The optimal conditions for maximum oil yield (22.27 g 100 g^{-1} on dry weight) correspond to temperature of 60°C, pressure of 120 bars and thickness cake of 1. 30 cm.

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