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Isolation and characterization of hydrophobic compounds from carbohydrate matrix of *Pistacia atlantica*

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ABSTRACT

The present work is focused on the optimization of hydrophobic compounds extraction process from the carbohydrate matrix of Iranian *Pistacia atlantica* seed at laboratory level using ultrasonic-assisted extraction. Response surface methodology (RSM) was used to optimize oil seed extraction yield. Independent variables were extraction temperature (30, 45, 60, 75 and 90 °C), extraction time (10, 15, 20, 25, 30 and 35 min) and power of ultrasonic (20, 40, 60, 80 and 100 W). A second order polynomial equation was used to express the oil extraction yield as a function of independent variables. The responses and variables were fitted well to each other by multiple regressions. The optimum extraction conditions were as follows: extraction temperature of 75 °C, extraction time of 25 min, and power of ultrasonic of 80 W. A comparison between seed oil composition extracted by ultrasonic waves under the optimum operating conditions determined by RSM for oil yield and by organic solvent was reported.

1. Introduction

Vegetable oils and fats are used in the food industry for the manufacture of a wide variety of products, ranging from margarines to chocolate or used directly as salad and cooking oils. The use of the oil in industry is determined by the composition of fatty acids, and this is highly dependent on its natural origin. The fatty acid composition of oils from vegetable sources varies depending on plant origin, genetic factors, ripening grade of fruits and specific climatic conditions (Davis & Poneleit, 1974; Velasco, Rojas-Barros, & Fernandez-Martinez, 2005).

The genus *Pistacia* is a member of the Anacardiaceae family and consists of 11 or more species (Kafkas, 2006; Turkelia & Kafkas, 2013; Zohary, 1952). Plants of this genus are primarily distributed in the Northern hemisphere and include both evergreen and deciduous species. However, pistachio, *Pistacia vera* L., is the only cultivated and commercially noteworthy species of this genus. It grows wild also in Algeria, Turkey, Morocco, France, Spain, Italy and Greece (Tingshuang, Jun, Avi, & Dan, 2008).

Pistacia atlantica is a tree located in North Africa, which can reach over 15 m in height and grows in arid and semi-arid areas (Benabid, 2000; Gourine et al., 2010), its vernacular name is "Butom". *P. atlantica* is valued because it is the source of mastic gum, exudates which strengthens gums, deodorizes breath, fights coughs, chills and stomach diseases (Bellakhder, 1997) (Fig. 1). Moreover, the galls of *P. atlantica* which are edible and sold in markets are used as an embalming gradient by rural habitants. Previous studies on *P. atlantica* deals with flavonoids (Mosharrafa, Kawashty, & Saleh, 1999), fatty acids and triglycerides (Benhassaini, Bendahmane, & Benchalgo, 2007; Farhoosh, Tavakoli, & Khodaparast, 2008; Yousfi, Nedjmi, Bellal, Ben-Bertal, & Palla, 2002; Yousfi, Nedjemi, Belal, & Ben Bertal, 2003; Yousfi, Nadjemi, Belal, Bombarda, & Gaydou, 2005), chemical composition of the oleoresin (Benhassaini, Bendeddouche, Mehdadi, & Romane, 2008; Delazar, Nazemyieh, Modarresti, & Afshar, 2002; Delazar, Reid, & Sarker, 2004), and chemical composition of the essential oils (Barrero et al., 2005; Mecherara-Idjeri, Hassani, Castola, & Casanova, 2008; Tzakou, Bazos, & Yannitsaros, 2007). Recently, a new hispolone compound has been isolated from the methanolic extract (Gourine et al., 2010; Yousfi et al., 2009). It also serves as a condiment in the north west of Tunisia and is used in the treatment of scabies, rheumatism and in the manufacture of anti-diarrhoea pills (Le Floc'h & Nabli, 1983).

Conventional solvent extraction is the most commonly used method to extract polysaccharides. Although CSE is simple and safe, high temperature and long extraction time of CSE lead to the degradation of polysaccharides and the decrease of the pharmacological activity of polysaccharides (Hromádková, Ebringerová, & Valachovic, 2002; Ying, Han, & Li, 2011). Recently some new extraction methods have been developed to improve the extraction

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Fig. 1. *Pistacia atlantica* seeds used in present work.

process, such as microwave-assisted extraction and ultrasound-assisted extraction.

Ultrasound is a form of energy generated by sound waves of frequencies that are too high to be detected by human ear, i.e. above 16 kHz (Jayasooriya, Bhandari, Torley, & D'Arcy, 2004). Ultrasound cavitations could result in the occurrence of micro streaming which is able to enhance heat and mass transfer (Yildirim, Durdu Oner, & Bayram, 2011). Ultrasound waves are characterized by the oscillating movement of medium particles and require a physical medium for propagation (Humphrey, 2007).

The acoustic cavitation in ultrasound-assisted extraction causes disruption of the cell walls, reduction of the particle size and enhancement on contact between solvents and targeted compounds (Rostagno, Palma, & Barroso, 2003). As ultrasound-assisted extraction has lower energy consumption, lower consumption of solvents, higher extraction efficiency and higher level of automation, ultrasound-assisted extraction is preferable to conventional solvent extraction. Ultrasound-assisted extraction has been used to extract the organic compounds from soil, animal and plant tissues (Hromádková & Ebringerová, 2003; Paniwnyk, Beaufoy, Lorimer, & Mason, 2001; Tor, Aydin, & Özcan, 2006; Ying et al., 2011).

The objective of this present study was to optimize the process for extraction of the oil from the seed of Iranian *P. atlantica* (OSPA), using response surface methodology (RSM), employing a five-level, five-variable central composite rotatable design (CCRD).

2. Materials and methods

2.1. Materials

P. atlantica seeds were purchased from local market in Mollasani, Khouzestan Province in Iran. All other chemicals and solvents used were of analytical grade and obtained from Merck Co., Germany.

2.2. Methods

2.2.1. Soxhlet extraction of OSPA

Traditional Soxhlet extraction was performed as described by Fadavi, Barzegar, and Azizi (2006), with slight modification. The oil from the *P. atlantica* seed powder (5 g) was extracted with 150 ml n-hexane for 3 h at 60 °C; the solvent was evaporated and recycled. Yield was expressed as the amount of oil obtained from a given weight of seeds. The Soxhlet extraction technique produced 5.59 ± 0.12 g of oil per 100 g of seeds, which was set at 100% oil recovery in comparison with the efficiency of ultrasonic extraction in our experiments. This oil was dried at 80 °C to a constant mass, and stored at 5 °C.

Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Factor level				
	−2	−1	0	1	2
Extraction temperature (°C)	30	45	60	75	90
Extraction time (min)	10	15	25	30	35
Power of ultrasonic (W)	20	40	60	80	100

2.2.2. Ultrasonic extraction of OSPA

The extraction of OSPA was performed according to Li, Qu, Zhang, and Wang (2012). The *P. atlantica* seed powder (5 g) seeds ground with a predetermined volume of n-hexane were placed in a 250-ml Erlenmeyer flask. Extractions were performed at required temperatures and for different time periods in an ultrasonic cleaning bath (KQ-400KDV, Kunshan, Zhejiang, China). Recycled water was provided to maintain a constant solution temperature. After being extracted, the mixture was filtered under vacuum through Whatman No. 1 paper (Whatman–Xinhua Filter Papers Co., Zhejiang, China), and the solvent was removed with a rotary vacuum evaporator at 50 °C. The amount of oil recovered was expressed as a percentage of that obtained through Soxhlet extraction (Zhang et al., 2009). Each extraction was performed in triplicate using three different samples.

2.3. Experimental design and statistical analysis

Response surface methodology (RSM) was adopted to evaluate how oil recovery is affected by independent variables, including the extraction temperature (°C, X_1), and extraction time (min, X_2), power of ultrasonic (W, X_3). Levels for each parameter were based on preliminary single-factor experiments in our laboratory (data not shown). All variables were fixed at five levels (Table 1). In all, 20 experiments were conducted according to a three-variable, five-level central composite rotatable design (CCRD). Our objective was to optimize the extraction process for greatest efficiency. Each experiment was performed in triplicate and the average oil recovery (%) was taken as response Y (%). This recovery was analyzed by quadratic polynomial regression equations, and the predicted Y was fitted into the equation with a multiple regression procedure. We developed the following model:

$$Y = \beta_o + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ii} X_i X_j \quad (1)$$

where Y is the predicted response; β_o, β_i, β_{ii}, and β_{ij} are regression coefficients of the intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are independent variables. Software from Design-Expert 8 (Stat-Ease, Inc., Minneapolis, MN, USA) was used for regression analysis of the experimental data and for obtaining the coefficients of our quadratic polynomial model. The quality of the fitted model was expressed by the coefficient of determination (R^2), and the statistical significance of the terms was also examined with an F-test.

2.4. Physicochemical properties of OSPA

The seed oils extracted by Soxhlet and ultrasonic extraction were analyzed for their physicochemical properties. Values for acid (969.17), iodine (993.20), peroxide (965.33), and saponification (920.160) were determined according to AOAC (1990) methods. All measurements were made in triplicate using three different samples.

Table 2
Response surface central composite design (uncoded) and results for extraction yield of OSPA.

Run	X_1 (°C)	X_2 (min)	X_3 (W)	Extraction yield (%)		
				Experimental	Predicted	Residual
1	45	15	80			
2	60	20	60	9.50	9.36	0.14
3	60	20	60	9.50	9.59	−0.09
4	60	20	60	12.10	11.99	0.11
5	60	20	60	9.10	9.16	−0.06
6	45	15	40	10.00	9.86	0.14
7	45	25	40	12.10	12.19	−0.09
8	45	25	80	13.00	12.99	0.01
9	60	20	60	9.20	9.08	0.13
10	60	20	100	12.00	12.18	−0.18
11	60	30	60	7.00	7.10	−0.10
12	60	10	60	12.80	12.75	0.05
13	75	15	40	8.80	8.88	−0.08
14	90	20	60	12.00	11.98	0.03
15	60	20	60	10.20	10.13	0.07
16	75	25	80	10.10	10.13	−0.03
17	30	20	60	10.10	10.13	−0.03
18	75	25	40	10.30	10.13	0.18
19	75	15	80	10.00	10.13	−0.13
20	60	20	20	10.00	10.13	−0.13

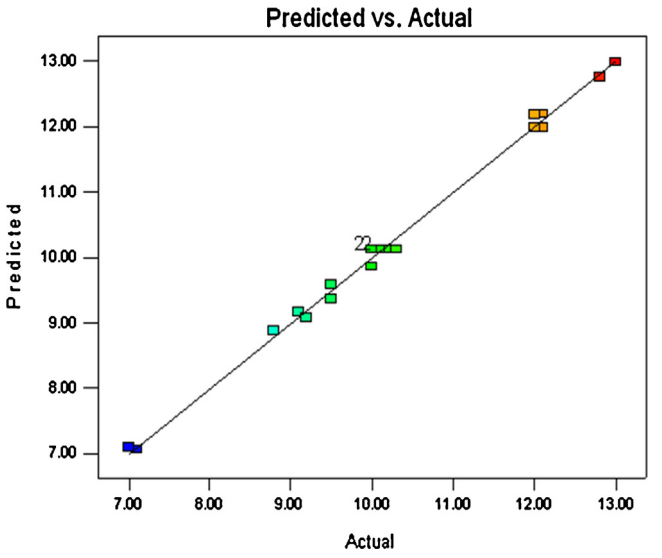

Fig. 2. Actual extraction yield vs. the predicted extraction yield under optimum extraction conditions.

3. Results and discussion

3.1. Fitting the model

The condition for the ultrasound extraction of OSPA was optimized using different variable (extraction temperature (°C, X_1), and extraction time (min, X_2), ultrasound frequency (HZ, X_3)) according to the central composite rotatable design (CCRD). The experimental and model-predicted values are listed in Table 2. The following regression equation was obtained for extraction yield of OSPA (Y):

$$Y(\%) = -1.9750 + 0.0583X_1 + 0.2675X_2 + 0.0712X_3 + 0.0003X_1X_2 - 0.0013X_1X_3 + 0.0012X_2X_3 + 0.0005X_1^2 - 0.0020X_2^2 + 0.0002X_3^2 \quad (2)$$

Table 3 presents the statistical significance of each coefficient through F- and p-values by a one-way analysis of variance (ANOVA). These data showed that the differences in three linear terms (X_1, X_2 and X_3), quadratic terms (X_{11}, and X_{33}), and two interaction terms (X_1X_3, X_2X_3) were significant ($p < 0.05$ or $p < 0.01$), while the other terms (X_1X_2 and X_{22}) were insignificant ($p > 0.05$).

Table 3
The significance of each response variable effect showed by using F and p values in the nonlinear second order model.

Variables	DF[a]	SS[b]	MS[c]	F-value	p-Value[d]
Linear effects					
X_1	1	9.6100	9.6100	468.7805	<0.0001
X_2	1	31.9225	31.9225	1557.1951	<0.0001
X_3	1	9.6100	9.6100	468.7805	<0.0001
Quadratic effects					
X_1^2	1	0.3929	0.3929	19.1638	0.0014
X_2^2	1	0.0629	0.0629	3.0662	0.1105 ns[e]
X_3^2	1	0.1414	0.1414	6.8990	0.0253
Interaction effects					
X_1X_2	1	0.0050	0.0050	0.2439	0.6321 ns[e]
X_1X_3	1	1.2800	1.2800	62.4390	<0.0001
X_2X_3	1	0.1250	0.1250	6.0976	0.0331

[a] Degrees of freedom.
[b] Sum of squares.
[c] Mean sum of squares.
[d] p values <0.05 were considered to be significant.
[e] Not significant.

Analysis of variance (ANOVA) was performed to investigate the adequacy of the suggested models and identify the significant factors. A significant lack of fit shows that the models failed to represent the data in the experimental domain at which points were not included in the regression. The R^2 and adj-R^2 values were 0.9962 and 0.9927, respectively (Table 4). A high R^2 indicates that the variation could be accounted for by the data satisfactorily fitting the model. However, a large value of R^2 does not always imply that the regression model is a good one. Adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, it is better to use an adj-R^2 to evaluate the model adequacy. The CV values were found to be 1.3975 for yield extraction of OSPA. Since CV is a measure expressing the standard deviation as a percentage of the mean, smaller values of CV give better reproducibility. In general, a CV higher than 10 indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model (Myers & Montgomery, 2002). The low PRESS 1.2309 value suggests for the adequacy of the fitted quadratic models for predictive applications (Table 4). Adequate precision measures the signal-to noise ratio. A ratio greater than 4 is desirable (Myers & Montgomery, 2002). For the proposed models, this value was 58.5230, a very good signal-to-noise ratio. All these statistical parameters show the reliability of the models. It seems that the extraction time is the most decisive working condition in the extraction yield of OSPA.

Fig. 2 shows that the polynomial regression model (Eq. (2)) was in good agreement with the experimental results. In this figure, the observed values are compared to the predicted values calculated from the model. The result suggests that the model used in this research was able to identify operating conditions for selective extraction of oil from seeds of Iranian *P. atlantica*.

3.2. Optimization of extraction conditions of OSPA

Figs. 3a and 4a illustrate the response surface and contour plots for the influence of extraction temperature and time on the extraction yield of OSPA at fixed ultrasonic power (60 W). Higher temperatures are beneficial to the solubility of the almond oil in the extracting solvent, and could accelerate the extracting process. A significant increase of the OSPA extraction yield was observed over the extraction temperature range (30–75 °C), the oil extraction yield reaching the maximum of around 13.00% at 75 °C. When

Table 4
Analysis of variance for the fitted models.

Source	Degree of freedom	Coefficient	Sum of square	Mean square	*F*-value	*p*-Value
Model	9		53.2045	5.9116	288.3713	<0.0001
Residual	10		0.2050	0.0205		
Lack of fit	5		0.1367	0.0273	2.0000	0.2325 ns
Pure error	5		0.0683	0.0137		
Total	19		53.4095			
R^2		0.9962				
Adj-R^2		0.9927				
CV		1.3975				
PRESS		1.2309				
Standard deviation		0.1432				
Adequate precision		58.5230				

the extracting time varied from 10 to 25 min, a remarkable increase of the OSPA recovery was observed. Beyond that time range, there was little difference observed in the recovery.

Figs. 3b and 4b show the response surface and contour plots for the influence of temperature and power of ultrasonic on the extraction yield of OSPA at fixed extraction time (25 min). At constant extraction time, an increase in temperature slightly increases the yield of OSPA. This could indicate that OSPA is easily extractable compound, even at low temperature. Ultrasonic sound had a significant effect on the extraction of oil. When the power was increased from 40 to 80 W, the yield of oil increased significantly and almost linearly from 10.8% to 12.5%, increase. Sonication is widely used for extraction of various substances from plant material and generates microscopic bubbles (Vilkhu, Mawson, Simons, & Bates, 2008; Vinatoru, 2001). The mechanism of the ultrasonic-assisted extraction has two main stages (Sahin & Samli, 2013):

1. Dissolution of soluble components on surfaces of the plant matrix occurs, which is called also as washing.
2. Mass transfer of the solute from the plant matrix into the solvent by diffusion and osmotic processes, which is known as slow extraction.

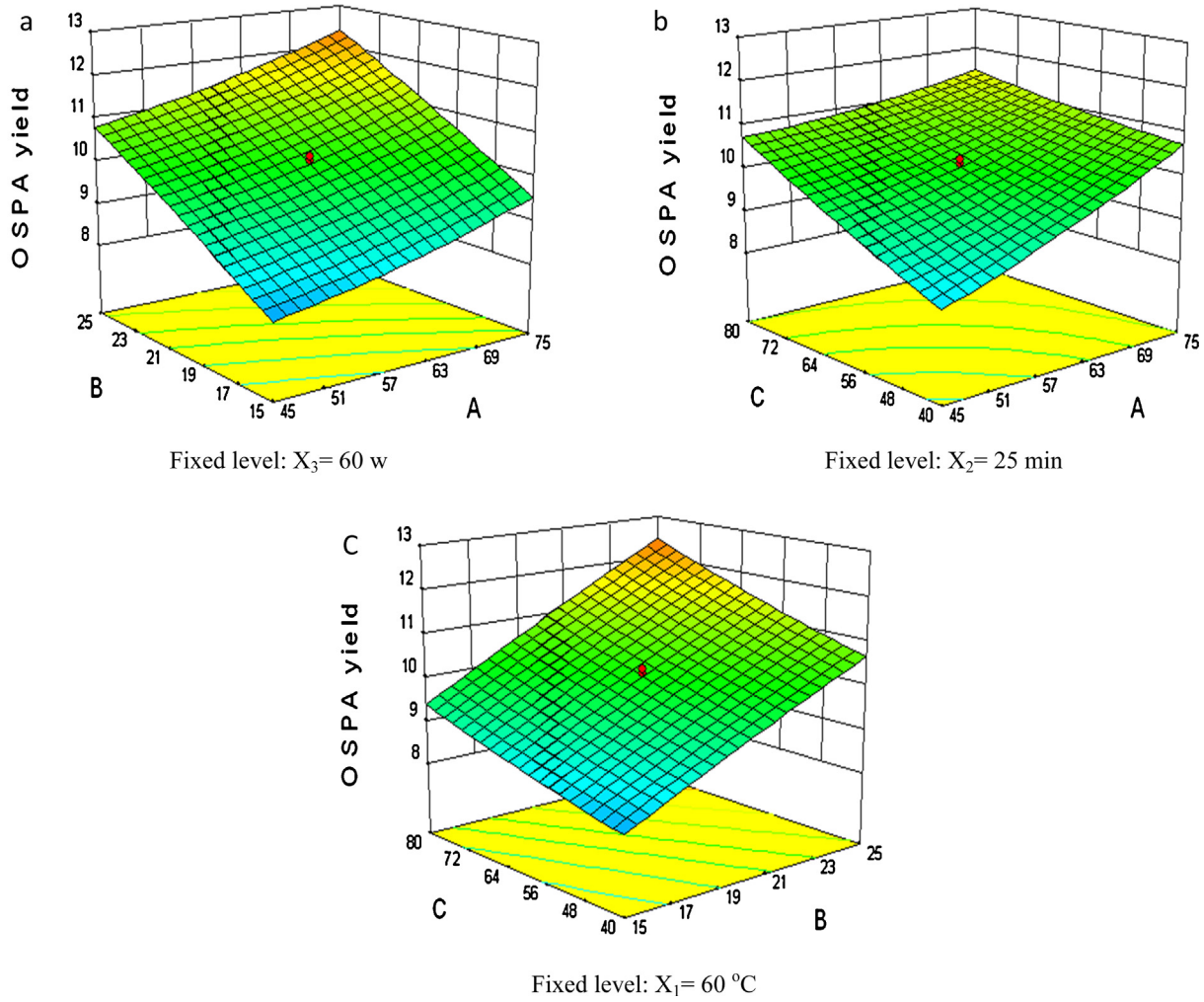

Fig. 3. Response surface (3-D) showing the effect of the extraction temperature (X_1), extraction time (X_2) and power of ultrasonic (X_3) on the extraction yield of OSPA (%).

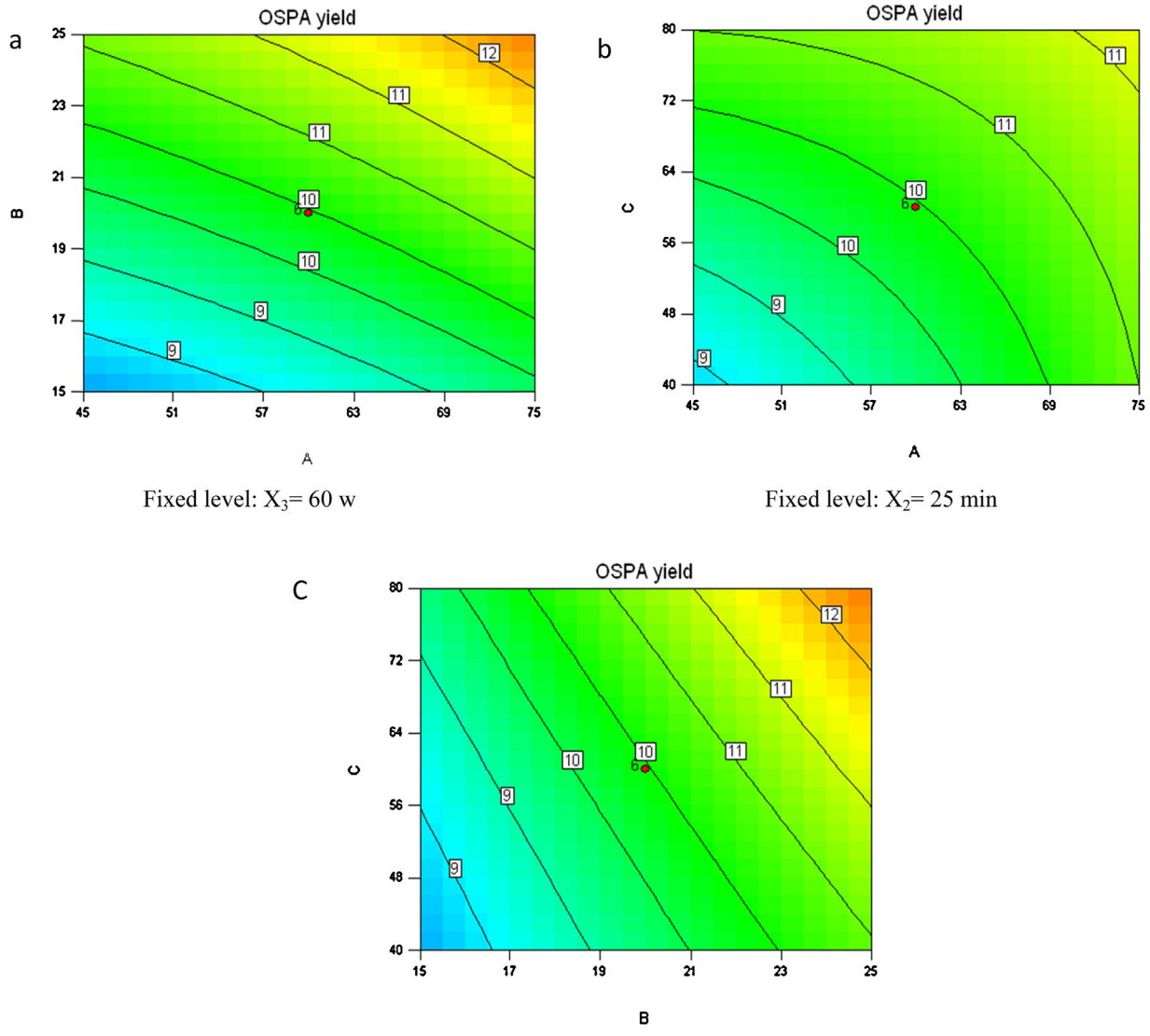

Fig. 4. Contour plots the effect of the extraction temperature (X_1), extraction time (X_2) and power of ultrasonic (X_3) on the extraction yield of OSPA (%).

This significantly accelerates the penetration of solvent into cells and the release of components from cells into the solvent, and simultaneously significantly enhances the mass transfer rate (Lou, Wang, Zhang, & Wang, 2010). As a result, the yield of OSPA increased proportionately with the increase in ultrasonic power (Fig. 1). Similar results have been reported by Sivakumar, Ravi Verma, Rao, and Swaminathan (2007).

Figs. 3c and 4c show the response surface and contour plots depicting the influence of extraction time and power of ultrasonic on the extraction yield of OSPA at fixed temperature (60 °C). It can be observed that at a fixed temperature the extraction yield of OSPA peaked at extraction time (25 min) and power of ultrasonic (80 W). The extraction with ultrasonic provides a more efficient means consuming less energy and time (Gutiérrez, Ratti, & Belkacemi, 2008). Results suggest that the longer the extraction time, the higher the extraction yield obtained for OSPA.

Fig. 5 shows the results of the optimization. According to this figure, the corresponding predicted response value under the optimum conditions for extraction yield of OSPA was 13.00%. From the optimization data, a combination of extraction temperature (75 °C), extraction time (25 min) and power of ultrasonic (80 W) was predicted for achieving the highest response for extraction yield of OSPA in the ranges studied (the highest value, 13.00%).

3.3. Model adequacy checking

Usually, it is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface likely give poor or misleading results (Li et al., 2007). The residuals from the least squares fit play an important role in judging model adequacy (Myers & Montgomery, 2002). By constructing a normal probability plot of the residuals, a check was made for the normality assumption, as given in Fig. 6. The normality assumption was satisfied as the residual plot approximated along a straight line. Fig. 7 presents a plot of residuals vs. the predicted response. The general impression is that the residuals scatter randomly on the display, suggesting that he variance of the original observation is constant for all values of extraction yield of OSPA. Both of the plots (Figs. 6 and 7) are satisfactory, so we conclude that the empirical model is adequate to describe the OSPA extraction yield by response surface.

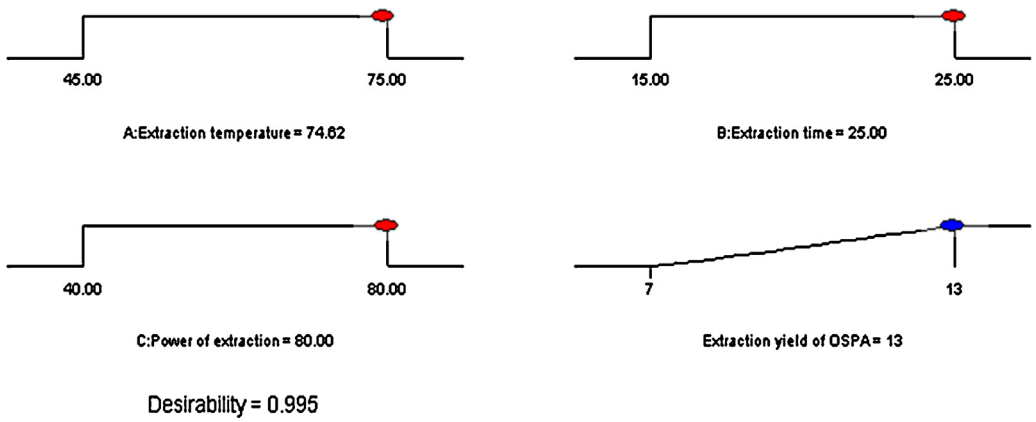

Fig. 5. Schematic representation of the optimum values of factors, response, and the corresponding levels.

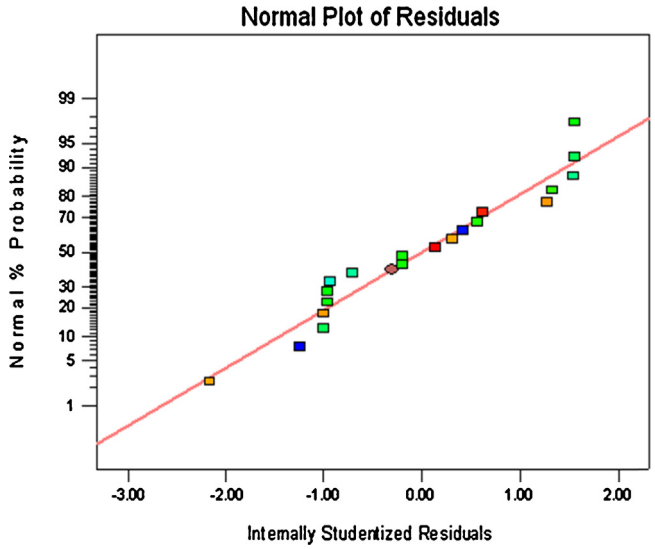

Fig. 6. Normal probability of internally studentized residuals.

Table 5
Predicted and experimental values of the responses at optimum conditions.

Optimum condition			Extraction yield of OSPA (%)	
Extraction temperature (°C)	Extraction time (min)	Power of ultrasonic (W)	Experimental	Predicted
75	25	80	13.26 ± 0.37	13.00 ± 0.14

3.4. Verification of predictive model

The optimum conditions identified from our model comprised an extraction temperature 75 °C; extraction time of 25 min and power of ultrasonic 80 W. In order to validate the adequacy of the model equations Eq. (2), a verification experiment was carried out under the optimal conditions. A mean recovery value of 13.26 ± 0.37% ($n = 4$) was obtained from the actual experiments, which approximated the value of 13.00% predicted by the model equation (Table 5). This good correlation between experimental and predicted values indicated that our response model (Eq. (2)) can be used to estimate oil extraction yield from the seeds of *P. atlantica*.

Table 6
Physicochemical properties of seed oil from *Pistacia atlantica*.

Parameter	Extraction method	
	Soxhlet	Ultrasonic
Acid value (mg of KOH per g of oil)	1.28 ± 0.05[a]	1.34 ± 0.04[a]
Peroxide value (meq of O_2 per kg of oil)	6.54 ± 0.14[a]	6.73 ± 0.19[a]
Iodine value (g of I2 per 100 g of oil)	108.16 ± 1.44[a]	106.39 ± 1.37[a]
Saponification value (mg of KOH per g of oil)	169.04 ± 1.51[a]	167.26 ± 1.73[a]

[a] Each value is the mean ± standard deviation of triplicate determinations. Within each row, means not followed by the same letter are significantly different at $p \leq 0.05$.

3.5. Physicochemical properties of seed oil

The OSPA obtained from Soxhlet and ultrasonic extractions were similar ($p < 0.05$) in their physicochemical properties (Table 6). The acid values were 1.28 ± 0.05 (Soxhlet) and 1.34 ± 0.04 (ultrasonic), were not significantly different ($p < 0.05$). The peroxide values were 6.54 ± 0.14 (Soxhlet) and 6.73 ± 0.19 (ultrasonic) mg of KOH per g of oil, which were within the allowable limits for edible oils. Their lower peroxide values showed that they had not deteriorated, and could be stored long-term (Li et al., 2012). Iodine contents

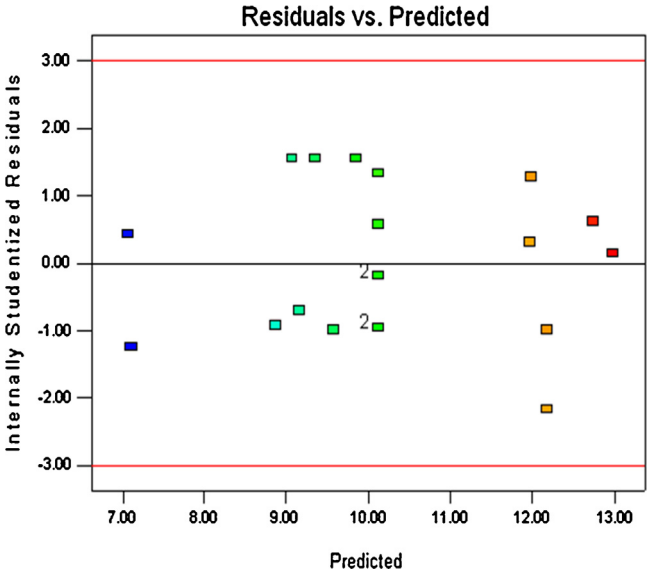

Fig. 7. Plot of internally studentized residuals vs. predicted response.

represented the degree of non-saturation, or the average number of double bonds, and the greater values of 108.16 ± 1.44 (Soxhlet) and 106.39 ± 1.37 (ultrasonic) indicated that the percentage of UFAs was high. Saponification values were 169.04 ± 1.51 (Soxhlet) and 167.26 ± 1.73 (ultrasonic) mg of KOH per g of oil. The results showed that the OSPA extracted by Soxhlet and ultrasonic had similar physicochemical properties.

4. Conclusion

In the present paper, the ultrasound-assisted extraction of oil from the seeds of *P. atlantica* was performed with a three-variable, five-level central composite design (CCRD) based on the RSM. Our experiments allowed us to optimize the conditions for obtaining seed oil via ultrasound-assisted extraction. A regression model was developed for predicting oil extraction yield. From this, we determined that the extraction time was the major contributing factor to the oil extraction. Also, it was revealed that the ultrasonic treatment did not affect the composition of the physicochemical properties of extracted oil.

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