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Deployment of response surface methodology to optimize recovery of dried dark fig (*Ficus carica* L., var. Azenjar) total phenolic compounds and antioxidant activity

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Abstract

Fig fruit (*Ficus carica* L.) is currently known by its high phenolic contents and antioxidant activity. Considering the importance of this fruit, we set as objective the conditions optimization for extracting total phenolic compounds (TPC) and antioxidant activity from dried dark fig using response surface methodology (RSM). The Box–Behnken design was used to investigate effects of three independent variables, solvent concentration (acetone/water 40–80%), temperature (25–65°C), and time (60–120 min), on the response. Second-order polynomial model was used for predicting the response. Regression analysis showed that about 99% of the variation was explained by the models. Response surface analysis showed that the optimal extraction parameters which maximized antioxidants extraction were 61.03% acetone, 105.12 min, and 46.16°C. Under optimal conditions, experimental values for TPC and antioxidant activity were 454.78 and 38.00 mg gallic acid equivalent (GAE)/100 g dry matter. The experimental values are in accordance with those predicted, indicating the suitability of the employed model and the success of RSM in optimizing the extraction conditions.

Introduction

Free radicals induce some oxidative damage to bio-molecules like lipids, nucleic acids, proteins and carbohydrates. Their damage causes aging, cancer and many other diseases. The harmful effects of these radicals can be reduced by the consumption of vegetables and fruits which provide numerous antioxidants such as phenolic compounds, carotenoïds, and vitamins E and C (Aruoma, 1994; Lako *et al*., 2008). Phenolic compounds are ubiquitous secondary metabolites in plants. They include a large group of biologically active compounds (above 8000 structures). Phenolics possess a wide spectrum of biochemical activities such as antioxidant and antimutagenic properties (Marinova *et al.*, 2005; Kang *et al*., 2012; Tan *et al.*, 2013).

Figs are infructescences of the fig tree (*Ficus carica* L.), a deciduous plant belonging to the Moraceae family. Fig fruit is an important crop worldwide (Solomon *et al.*, 2006), which consumed fresh, dried or used as jam (Oliveira *et al.*, 2009). In 2011, one million tons were produced worldwide. Algeria is the third most important producer of figs with 150,000 tons (FAO, 2011). Figs are an excellent source of phenolic compounds and present a high antioxidant activity which can prevents several diseases (Vinson *et al*., 1999; Chawla *et al*., 2012; Debib *et al*., 2013).

Fig is a highly perishable fruit, thus nearly all the world production is preserved in dried form (Owino *et al*., 2004). Among the commonly consumed fruits and beverages, dried fig is one of food with highest content of polyphenols. Antioxidants of this fruit can enrich lipoproteins of plasma and protect them from oxidation. This fruit can also produce a significant increase of plasma antioxidant capacity for a few hours after consumption (Vinson *et al*., 1999; Vinson *et al.*, 2005).

Extraction is the first step in isolation of antioxidants from plant material and plays a crucial stage in quantification and identification of these compounds. Optimization of antioxidants extraction may be achieved by either empirical or statistical methods and is essential for commercial application of the bioactive compounds extraction process (Rodrigues *et al.*, 2008; Annegowda *et al.*, 2012). Response surface methodology (RSM) is a statistical experimental protocol used in mathematical modeling (Triveni *et al.*, 2001; Gong *et al.*, 2012). This method reduces the experimental essays, improving the statistical interpretation possibility and indicating the interaction between variables (Tsapatsaris *et al.*, 2004; Yim *et al*., 2012). Using statistical software, for example JMP (SAS Institute Inc.), the RSM can give a mathematical equation. Moreover, it is helpful to calculate the response value when different levels of variables are set. Box–Behnken design is a widely

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used protocol in response surface methodology (Yang *et al*., 2008; Rao, 2010). Besides, the combination of processing parameters can be optimized through this technique and a high efficiency may be obtained. In order to study the influence of three parameters that affected the extraction of antioxidants from fig, we fixed as objective in this investigation the optimization of extraction conditions (solvent concentration, temperature, and time) of total phenolic contents and antioxidant activity from dried dark fig using RSM methodology.

Materials and Methods

Sample preparation

The dried dark fig variety, harvested in the region of Bejaia (North of Algeria), was used in this study. The dark fig, locally known as Azenjar, presents a dark-purple skin and red pulp with fresh and dried weight of 46.65 and 24.73 g, respectively. The sample (about 1 kg) was randomly harvested and sun dried following the traditional method. The fruits were arranged in a single layer on a plate and exposed to sunlight (with an average daily temperature of 27.8°C). During night the fruits were kept in the room, at room temperature, to avoid receiving the night humidity. Figs were considered dried when moisture content was lower than 30%. The drying process was completed within one week. The dried sample was cut into small pieces, lyophilized (Alpha1-4 LDplus lyophilizer, Christ, Osterode, Germany) then grinded (A11 basic grinder, Ika, Staufen, Germany). The obtained powder was stored at –20°C prior to analysis.

Extraction process

The initial step of the preliminary experiment was to select an appropriate extraction medium for dried dark fig antioxidants. Effects of solvent nature (acetone, ethanol, methanol, and water), solvent concentration (20–80%), extraction temperature (25–70°C), extraction time (0.5–4 h), and sample to solvent ratio (1/25–1/100) were tested in our previous study (Bachir bey *et al*., 2013) and the conclusions were used in this study to optimize antioxidants extraction with RSM methodology.

An aliquot of lyophilized dark fig (0.67 g) was placed in a 100-mL glass vial with 50 mL of solvent containing variable amounts of acetone/water. Extractions were carried out under magnetic stirring at 400 rpm, at different temperature and time (Table 1). The extracts were separated by centrifugation at 5000 rpm (NF 200, Nüve, Turkey) for 10 min.

Table 1. Factors and levels for response surface methodology, Box–Behnken design matrix (in coded and uncoded level of three variables), experimental data and predicted values for three-level-three-factor response surface analysis

Run	Variable levels[a]			TPC[b]		Antioxidant activity[b]	
	x_1	x_2	x_3	Observed	Predicted	Observed	Predicted
1	80 (+1)	90 (0)	25 (–1)	379.18	383.83	30.18	30.71
2	80 (+1)	90 (0)	65 (+1)	389.68	389.35	32.89	32.55
3	60 (0)	120 (+1)	25 (–1)	446.92	446.61	36.82	36.46
4	40 (–1)	120 (+1)	45 (0)	386.4	386.37	32.616	32.63
5	40 (–1)	90 (0)	25 (–1)	356.18	356.52	29.26	29.60
6	40 (–1)	60 (–1)	45 (0)	342.1	346.44	28.92	29.10
7	60 (0)	90 (0)	45 (0)	451.68	448.71	37.22	37.25
8	60 (0)	60 (–1)	25 (–1)	375.54	370.86	32.71	32.19
9	60 (0)	60 (–1)	65 (+1)	430.94	431.25	35.49	35.85
10	80 (+1)	60 (–1)	45 (0)	361.76	361.79	31.56	31.54
11	80 (+1)	120 (+1)	45 (0)	398.98	394.64	32.83	32.66
12	60 (0)	90 (0)	45 (0)	442.08	448.71	37.25	37.25
13	60 (0)	90 (0)	45 (0)	452.36	448.71	37.28	37.25
14	60 (0)	120 (+1)	65 (+1)	423.6	428.28	35.71	36.23
15	40 (–1)	90 (0)	65 (+1)	397.7	393.05	31.72	31.19

[a] x_1, Solvent concentration (%); x_2, Time (min); x_3, Temperature (°C)
[b] TPC and antioxidant activity were expressed in mg GAE/100 g DM of dried dark fig.

Total phenolic content

Total phenolic content of extracts was assessed using the Folin-Ciocalteu reagent method (Singleton and Rossi, 1965). Folin-Ciocalteu reagent (750 µL) and sodium carbonate (400 µL, 7.5% w/v) were added to 200 µL of extract. The absorbance at 720 nm was measured in a UV–Vis spectrophotometer (UVmini 1240, Schimadzu, Suzhou Jiangsu, China) after 60 min of incubation. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per 100 grams of dry matter (DM) (Abs = 13 GAE; $R^2 = 0.99$).

Determination of antioxidant activity

The scavenging capacity for the radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used to determine the antioxidant activity according to Molyneux (2004). An aliquot (200 µL) of the extract was added to 1 mL of methanolic DPPH solution (60 µM). The decolorizing process was recorded at 515 nm after 30 min of reaction. The scavenging activity of fig extracts was calculated using a calibration curve achieved with gallic acid (Abs = −49.75 GAE + 0.46; $R^2 = 0.99$) and expressed as mg GAE/100 g DM.

Experimental design

One of the common experimental designs used for engineering purposes is a Box–Behnken design that includes three variables and three factorial levels (Radojković *et al.*, 2012). The independent variables used in this study were acetone concentration (x_1, %, v/v), extraction temperature (x_2, °C), and time (x_3, min) while response variable were TPC and antioxidant activity. Coded and uncoded levels of

Table 2. Regression coefficient, standard error, and Student's t-test results of response surface for TPC and antioxidant activity

Parameter	Estimate	Std Error	*t* Ratio	Prob>|*t*|
TPC				
Intercept	448.707	3.571	125.670	<0.0001*
x_1	5.903	2.187	2.700	0.0428*
x_2	18.195	2.187	8.320	0.0004*
x_3	10.513	2.187	4.810	0.0048*
$x_1.x_2$	–1.770	3.092	–0.570	0.5918
$x_1.x_3$	–7.755	3.092	–2.510	0.054
$x_2.x_3$	–19.680	3.092	–6.360	0.0014*
$x_1.x_1$	–57.481	3.218	–17.860	<0.0001*
$x_2.x_2$	–18.916	3.218	–5.880	0.002*
$x_3.x_3$	–10.541	3.218	–3.280	0.0221*
Antioxidant activity				
Intercept	37.250	0.332	112.090	<0.0001*
x_1	0.618	0.204	3.040	0.0289*
x_2	1.162	0.204	5.710	0.0023*
x_3	0.855	0.204	4.200	0.0085*
$x_1.x_2$	–0.607	0.288	–2.110	0.0889
$x_1.x_3$	0.063	0.288	0.220	0.8367
$x_2.x_3$	–0.973	0.288	–3.380	0.0197*
$x_1.x_1$	–4.969	0.300	–16.590	<0.0001*
$x_2.x_2$	–0.799	0.300	–2.670	0.0444*
$x_3.x_3$	–1.268	0.300	–4.230	0.0082*

* Values statistically significant at $p < 0.05$.

the independent variables and the experimental design were given in Table 1. Coded value 0 stands for centre point of the variables and was repeated for experimental error. Factorial points were coded as ± 1.

Statistical analysis and verification of model

All experimental data were centered by using three measurements. The response surface regression procedure of JMP 10 (statistical analysis system Inc., SAS) software was used to analyze the experimental data. Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows equation:

$$y = a_0 + \sum_{i=1}^{3} a_i x_i + \sum_{i=1}^{3} a_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} a_{ij} x_i x_j \quad (i \neq j) \qquad (1)$$

where a_0, a_i, a_{ii}, and a_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and x_i, and x_j are the independent variables.

Fischer's test was used for determination of the type of the model equation, while Student's t-test was performed for the determination of statistical significance of regression coefficients. Optimal conditions for the extraction of TPC and antioxidant activity from dried dark fig depended on solvent composition, extraction temperature, and extraction time were obtained using the predictive equations of RSM. The experimental and predicted values were compared in order to determine the validity of the model.

Results and Discussion

Analysis of the model

The optimization of antioxidants extraction from dried dark fig was based on maximizing TPC extraction and antioxidant activity. In order to reduce the number of parameters to be tested, several parameters were already tested in a wider range prior to RSM optimization (Bachir bey *et al*., 2013).

The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique and were displayed in Table 2. It was evident that the three linear, the three quadratic, and the interaction (x_2 x_3) parameters were significant at the level of $p < 0.05$, whereas the two other parameters (x_1 x_2 and x_1 x_3) were not significant. The fitted quadratic model for TPC and antioxidant activity in coded variables were given in equations 2 and 3.

$$TPC = 448.707 + 5.903x_1 + 18.195x_2 + 10.513x_3 - 1.770x_1x_2 - 7.755x_1x_3 - 19.680x_2x_3 - 57.481x_1^2 - 18.916x_2^2 - 10.541x_3^2 \qquad (2)$$

$$Antioxidant\ activity = 37.250 + 0.618x_1 + 1.162x_2 + 0.855x_3 - 0.607x_1x_2 + 0.063x_1x_3 - 0.973x_2x_3 - 4.9691x_1^2 - 0.799x_2^2 - 1.268x_3^2 \qquad (3)$$

Table 3 presents the results of fitting quadratic model of data. Results of variance analysis (ANOVA) indicate that the contribution of quadratic model was significant ($p < 0.05$) for response of the dependent variables, TPC and antioxidant activity. The ANOVA analysis indicates a good model performance with the correlation coefficient (R^2) values of 0.990 and 0.986 for TPC and antioxidant activity, respectively. These can explain 99.0 and 98.6% of calculated model. The statistical analysis gave high significant level, attesting the goodness of fit of the model in case of the TPC ($p = 0.0002$) and antioxidant activity ($p = 0.0004$). The results indicated that the model could work well for the prediction of the two studied parameters from dried dark fig.

The three studied parameters, solvent concentration, time, and temperature, were found to have positive linear effect on TPC extraction and antioxidant activity. The quadratic effects of the three parameters and the interaction between time-temperature influenced negatively both TPC extraction and antioxidant activity. However, interaction terms between solvent concentration-time and solvent concentration-temperature were found to have no effects.

Analysis of response surfaces

The best way of expressing the effect of any independent variable on the TPC extraction and

Table 3. ANOVA table for the effect of acetone concentration, time, and temperature on TPC extraction and antioxidant activity

Source	DF[a]	Sum of Squares	*F* Ratio	Prob > *F*
TPC				
x_1	1	278.716	7.287	0.0428*
x_2	1	2648.464	69.247	0.0004*
x_3	1	884.101	23.116	0.0048*
$x_1.x_2$	1	12.532	0.328	0.5918
$x_1.x_3$	1	240.560	6.290	0.054
$x_2.x_3$	1	1549.210	40.506	0.0014*
$x_1.x_1$	1	12199.555	318.971	<0.0001*
$x_2.x_2$	1	1321.140	34.543	0.002*
$x_3.x_3$	1	410.249	10.726	0.0221*
Model	9	18689,938	54,297	0,0002*
Error	5	191,233		
Total model	14	18881,171		
$R^2 = 0.990$				
Adj. $R^2 = 0.972$				
Antioxidant activity				
x_1	1	3.055	9.222	0.0289*
x_2	1	10.802	32.603	0.0023*
x_3	1	5.848	17.652	0.0085*
$x.x_2$	1	1.471	4.441	0.0889
$x_1.x_3$	1	0.016	0.047	0.8367
$x_2.x_3$	1	3.783	11.418	0.0197*
$x_1.x_1$	1	91.176	275.194	<0.0001*
$x_2.x_2$	1	2.359	7.119	0.0444*
$x_3.x_3$	1	5.939	17.925	0.0082*
Model	9	119,534	40,088	0,0004*
Error	5	1,657		
Total model	14	121,191		
$R^2 = 0.986$				
Adj. $R^2 = 0.962$				

[a] Degrees of freedom.
* Values statistically significant at $p < 0.05$.

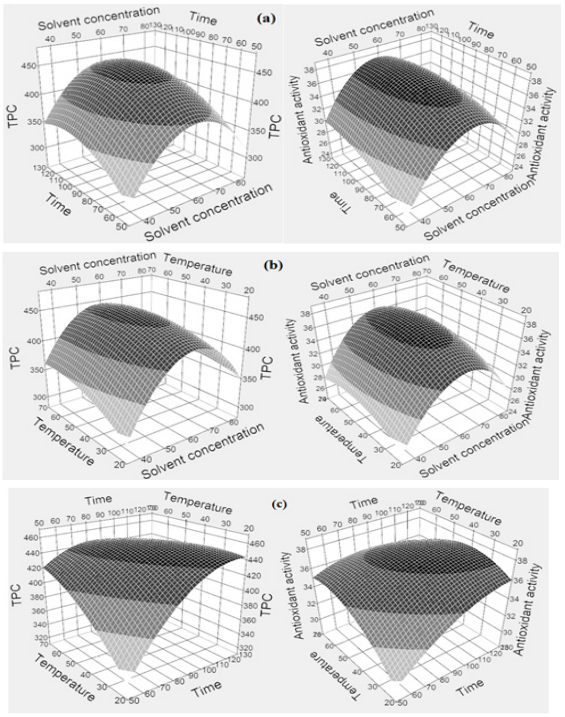

Figure 1. Response surface plots showing the effects of (a) solvent concentration (%) and time (min), (b) solvent concentration (%) and temperature (°C), and time (min) and temperature (°C) on TPC extraction and antioxidant activity of dried dark fig.

antioxidant activity was to generate surface response plots of the model, which were done by varying two variables within the experimental range under investigation and holding the other variable at its central level (0 level).

Figure 1a is the three dimensional plot showing the effects of solvent concentration (x_1) and time (x_2) on the TPC extraction and antioxidant activity of dried dark fig. It can be observed that solvent concentration exerts higher effects than time. Therefore, polarity played an important on the extraction of antioxidants. The increase of acetone concentration in the solvent caused a decrease in its polarity, which favored the extraction of less polar components (Cheok *et al*., 2012). Besides, increase of acetone concentration promoted the breakdown of cell membrane that enhances the permeability of the solvent into the solid matrix (Zhang *et al*., 2006; Vatai *et al.*, 2009). Nevertheless, at a very high acetone concentration the resulting polarity was inappropriate for the extraction of antioxidants from dried dark fig.

Acetone/water mixtures were good solvents for polar antioxidants extraction and more useful for phenolic extraction from different matrices (Kallithraka *et al.*, 2003; Luximon-Ramma *et al.*, 2003; Bachir bey *et al*., 2013). As shown previously by several authors, acetone/water mixtures were the best extraction solvents. Al-Farsi and Lee (2008) reported that 50% acetone was the most efficient solvent for phenolic extraction from date seeds and Chaalal *et al.* (2012) showed that 75% acetone was selected as the best solvent for antioxidants extraction from prickly pear seeds.

The effects of solvent concentration (x_1) and temperature (x_3) on TPC extraction and antioxidant activity from dried dark fig were showed in Figure 1b. It indicates that solvent concentration affects more considerably the antioxidants extraction than temperature.

According to Figure 1c, it was observed that both time and temperature influenced simultaneously TPC extraction and antioxidant capacity. These latter increased until time and temperature reach 105.89 min and 46.30°C, respectively. The mass transfer from plant material to solvent was related to time and temperature.

The extraction time was an important factor that influenced TPC extraction and, hence, antioxidant activity. The mass transfer increase with time until the maximum of extraction was achieved. However, long time extraction affects negatively the antioxidant extraction, that probably due to decomposition of the active compounds during the prolonged extraction time. Furthermore, undesirable components, such as proteins and polysaccharides, can be dissolved (Shi *et al*., 2003).

The high temperature accelerates the diffusion,

thus increasing the extraction (Kassama *et al.*, 2008). For long time of extraction under high temperature, however, the negative quadratic effect became significant. Higher extraction temperature beyond 46.30°C did not showed a significant improvement of TPC extraction and antioxidant activity. This may be attributed to the thermal degradation of antioxidants at high temperature conditions which was favored by long time of extraction (Prommuak *et al*., 2008), indicating that extracts contained a heat sensitive compounds.

Determination and experimental validation of the optimal conditions

In order to verify the predictive capacity of the model, the optimal conditions were determined using the maximum desirability and were used for an extraction test for TPC and antioxidant activity. Results of optimal conditions to obtain the highest extraction of phenolics from dried dark fig, as well as maximum antioxidant activity, were acetone concentration of 61.03%, temperature of 46.16°C, and time extraction of 105.12 min. Under optimal conditions, the experimental values were 454.78 ± 8.53 and 38.00 ± 0.43 mg GAE/100 g DM for TPC and antioxidant activity, respectively. These experimental results were in agreement with the predicted values for TPC (453.16 mg GAE/100 g DM) and antioxidant activity (37.65 mg GAE/100 g DM).

Conclusion

High correlation of the mathematical model indicated that a quadratic polynomial model may be employed to optimize the solid–liquid extraction of antioxidants from dried dark fig. From response surface plots, all the three studied factors (acetone concentration, temperature, and time) significantly influenced TPC and antioxidant activity of fig extracts. The experimental values were found to be in agreement with the predicted ones and clearly indicated the suitability of the developed quadratic models. These results confirm the predictability of the model for the extraction of TPC and antioxidant activity from dried dark fig in the experimental conditions used.

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