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Ultrasonic-assisted extraction of total phenolic contents from *Phoenix dactylifera* and evaluation of antioxidant activity: statistical optimization of extraction process parameters

Fatiha Benkerrou[1] · Mostapha Bachir bey[1] · Meriem Amrane[1] · Hayette Louaileche[1]

Abstract

The present study aimed to optimize extraction conditions of total phenolic contents (TPC) and evaluation of antioxidant activity (AA) of date palm fruit. The Box–Behnken design was used to study effects of three independent variables, acetone concentration (40–80%), sonication amplitude (50–100%), and extraction time (15–35 min). The statistical optimization revealed that extraction with acetone concentration of 66.71% (v/v) for 29.58 min with sonication amplitude of 64.78% were the best combination of these variables. The corresponding experimental values for both TPC and AA were 725.33 and 39.61 mg GAE/100 g DM of date fruit, respectively. Predicted values were in close agreement with experimental ones. Elaborated models were significant ($P > 0.05$) with high regression coefficients ($R^2 \geq 0.9$) and insignificant lack of fits that confirm the validity and success of both MRS models to optimize extraction conditions of antioxidants date fruit.

Keywords Date fruit · Ultrasound · Phenolic compounds · Antioxidant activity · Optimization

Introduction

Phoenix dactylifera, commonly known as date palm, constitutes the basis of economy for the people living in Algerian Sahara. Date fruit is well known for its nutritional values and numerous health properties, and considered as an excellent source of rapid energy. This fruit was listed in folk remedies and recommended for the treatment of various infection diseases and cancer [1–3].

Several studies revealed that date extracts have numerous biological properties including anti-inflammatory, nephro-protective, immunostimulant, and neuroprotective activities [4, 5]. The protective effects of date fruit against the previously mentioned diseases are attributed to vitamin C, trace elements, and to bioactive compounds such as carotenoids, sterols, tannins, isoflavones, lignans, flavonoids and other polyphenols [1]. In addition, these phytochemicals possess the potential to scavenge free radicals responsible for oxidative stress [6].

In order to benefit from these bioactive compounds, an efficient extraction is essential. In this context, many factors can influence extraction of these compounds such as extraction method, solvent, temperature, time, etc. For date fruit, numerous procedures for phenolic compounds extraction were used. The most solvent used for the extraction were methanol (20–99%), ethanol (50%), and acetone (60–70%) with variable extraction times (30 min to 48 h) using generally maceration [7–10].

Ultrasound is one of the emerging technologies that was used to maximize quality, minimize processing, and guarantee the safety of food product. This technique can dramatically enhance mass-transfer in various foods and promotes homogenization and extraction of intracellular compounds [11]. Ultrasound-assisted extraction (UAE) improves significantly extraction of bioactive compounds from vegetal matrices [12]. Its mechanical effects provide a greater penetration, mainly through the phenomenon of cavitation [13], ultrasound improve productivity, yield, selectivity, and economize time of processing [14].

Experimental design methodology such as response surface methodology (RSM) is based on the development of

✉ Mostapha Bachir bey
   bachirbeymustapha@gmail.com

[1] Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaïa, Algeria

series of experimental tests that aimed to improve, optimize, and evaluate effects of variables and their interactions.

There is no data reported about statistical optimization of extraction conditions of phenolic compounds from date fruit with MRS using UAE. Consequently, the aim of this study is to optimize the UAE conditions (acetone concentration, extraction time, and sonication amplitude) of total phenolic content (TPC) and antioxidant activity (AA) of date fruit using RSM.

Materials and methods

Chemicals

Folin–Ciocalteu reagent was from Biochem Chemopharma (Montreal, Quebec); 1,1-diphenyl-2-picrylhydrazyl radical was from Sigma Aldrich (Steinheim, Germany), gallic acid was from Sigma Aldrich co (St. Louis, MO, USA), sodium carbonate was from Biochem Chemopharma (Georgia, USA), acetone (99.78% purity), ethanol (99.7% purity) and methanol (99.70%) were from VWR Prolabo (Fontenay-sous-Bois, France).

Sample preparation

The mature date fruits of *Litim* cultivar were harvested in M'zab oasis of Ghardaia department (Algeria). This cultivar was characterized by its dark brown color with dimensions of 3.6 cm (length) $\times$ 1.9 cm (width) and the moisture test indicate that this fruit have a water content of 20.93%. After harvest, the dates were transported to the laboratory. Mature fruit with uniform size free of physical damage, insect injury, and fungal infection were selected. The fruit pulp was manually separated from seeds and crushed by a hand grinding mill.

Optimization of extraction conditions

Before application of experimental design, a preliminary study was carried out using sequential methodology that consists to vary one factor at a time and let others constant. This step was performed in order to select factors that affect extraction of antioxidants and to determine ranges of influencing factors. The tested factors were solvent type (ethanol, methanol, acetone, and water), solvent concentration (20–100%), sample/solvent ratio (1/100, 2/100, and 3/100), extraction time (5–35 min), and sonication amplitude (25–100%). The statistical analysis of preliminary results revealed that three variables (acetone, sonication amplitude, and extraction time) were selected as significant parameters and used in order to optimize extraction conditions of date antioxidants using RSM.

Extraction procedure

An aliquot of date paste was weighed into a test tube and 20 ml of solvent were added. The extraction was carried out by an ultrasonicator equipped with a probe. The used Sonicator (Sonics Vibracell VCX 130 PB, USA) was characterized by power output of 130 W and a frequency of 20 kHz and equipped with a titanium alloy probe with dimensions of 138 mm (length) $\times$ 3 mm (diameter). The probe was introduced into the tube containing sample and extraction solvent at a depth of 5 cm. To prevent possible evaporation of extraction solvent, a rubber hatch was set on sonicator probe to close the tube and prevent solvent evaporation. Sonication was performed in an ice bath in order to keep the extract in low temperature (the temperature doesn't exceed 22 °C during all extractions). Subsequently, the mixture was centrifuged at 4000$\times$g (NF 200, Nuve, Turkey) for 5 min and resulted extract was then filtered.

Total phenolic contents

The extract (200 µl) was mixed with 750 µl of Folin–Ciocalteu reagent, 400 µl of sodium carbonate (5%) were added after 5 min. After 60 min, the absorbance was measured with a UV–Vis spectrophotometer (UViline 9400, Secomam, Alés, France) at 720 nm. TPC was expressed as milligrams of gallic acid equivalent (GAE) per 100 g dry mater (DM) of date fruit [15].

Antioxidant activity

The extract (100 µl) was added to 1 ml of a methanolic DPPH solution (80 µM). After 30 min, absorbance was measured at 515 nm. The scavenging activity of date extracts was calculated using a calibration curve prepared with gallic acid and results were expressed as mg GAE/100 g DM of date fruit [16].

Experimental design

RSM with Box–Behnken design was used to optimize parameters affecting extraction of TPC and AA. In the current study, fifteen experiments were carried out with different ranges of acetone concentration (40, 60, and 80%), sonication amplitude (50, 75, and 100%), and extraction time (15, 25, and 35 min) as indicated in Table 1. Coded value 0 stands for centre point, $+1$ for the maximal value, and -1 for the minimal value.

Data analysis

In order to carry out the experimental design and statistical analysis, the response surface regression was studied

Table 1 Box–Behnken design matrix and experimental and predicted data

Run	Variable levels			Total phenolic content (mg GAE/100 g DM)		Antioxidant activity (mg GAE/100 g DM)	
	x_1	x_2	x_3	Experimental	Predicted	Experimental	Predicted
1	60 (0)	100 (+)	35 (+)	506.59	506.50	39.36	37.50
2	60 (0)	50 (−)	35 (+)	708.64	671.64	41.58	39.80
3	40 (−)	75 (0)	35 (+)	416.21	454.72	27.01	29.68
4	80 (+)	75 (0)	15 (−)	541.72	503.21	32.26	29.59
5	60 (0)	75 (0)	25 (0)	683.08	702.09	39.19	39.67
6	80 (+)	100 (+)	25 (0)	565.61	567.12	36.27	37.16
7	60 (0)	50 (−)	15 (−)	507.52	507.61	33.35	35.21
8	60 (0)	100 (+)	15 (−)	503.38	540.38	26.41	28.19
9	80 (+)	50 (−)	25 (0)	595.21	633.63	35.22	36.02
10	80 (+)	75 (0)	35 (+)	670.94	669.52	37.94	38.91
11	40 (−)	50 (−)	25 (0)	521.25	519.74	35.85	34.96
12	60 (0)	75 (0)	25 (0)	696.16	702.09	41.67	39.67
13	60 (0)	75 (0)	25 (0)	727.03	702.09	38.16	39.67
14	40 (−)	75 (0)	15 (+)	489.45	490.87	26.07	25.10
15	40 (−)	100 (+)	25 (0)	492.31	453.89	25.29	24.49

x_1, solvent concentration (%); x_2, sonication amplitude (%); x_3, time (min)

using JMP 10 (statistical analysis system Inc., SAS) software. Experimental data were fitted to a second order polynomial model and expressed by Eq. (1):

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

a_0, a_i, a_{ii}, a_{ij} are regression coefficients for intercept, linear, quadratic, and interaction terms, respectively; x_i and x_j are independent variables; n was the number of optimized factors.

Fischer and Student t tests were performed for the determination of model equation and for determination of statistical significance of regression coefficients.

Validation of models

From elaborated models, JMP software calculates the optimal conditions of factors (acetone concentration, sonication amplitude, and extraction time) for extraction of total phenolic compounds and AA. In order to conclude on validity of the two established models, obtained optimal predicted conditions were subjected to experimental test. Three independent extractions were performed using the optimal conditions found by the prediction profiler of JMP software. The values of TPC and AA obtained experimentally were compared with the theoretical values calculated by the software using Student t test.

Results and discussion

Model analysis

Combination of the three independent variables (acetone concentration, sonication amplitude, and extraction time) and the corresponding response values obtained in different experiments were presented in Table 1. It indicated that TPC was ranged from 416.21 to 727.03 mg GAE/100 g DM, while AA varied from 25.29 to 41.67 mg GAE/100 g DM. Experimental results agree with predicted values for both responses.

Two parameters were optimized in this study, TPC and AA. The measurement of TPC was carried out by Folin–Ciocalteu reagent, which based on the electron transfer from an antioxidant to phosphotungstic and phosphomolybdic acids mixture. This method is not very specific to phenolic compounds because other molecules can give a positive reaction with Folin–Ciocalteu reagent such as reducing sugars and some metals like iron. The AA used in this study was a hydrogen atom transfer (HAT) method that measures the ability of an antioxidant to quench free radical by HAT. This type of methods is fast and is characterized by relatively low activation energy [17]. Moreover, the potentiometric measurement (Potentiostat/Galvanostat PGP201 VoltaLab, Copenhagen, Denmark) of Folin–Ciocalteu and DPPH solutions showed that redox potentials were 512 and 434 mV, respectively. This indicates that oxidizing power of Folin–Ciocalteu reagent was greater than that of DPPH,

which explains the high values of phenolic compounds compared to AA.

Table 2 showed the influence of acetone concentration, sonication amplitude, extraction time, and interactions between these parameters on TPC and AA. Phenolics extraction was influenced by positive linear term of acetone concentration and negative quadratic terms of the three independent variables, while AA, was influenced by positive linear terms of acetone concentration and extraction time as well as negative quadratic term of acetone concentration. However, other linear and quadratic terms as well as interactions between factors [acetone concentration–sonication amplitude (x_1–x_2), acetone concentration–extraction time (x_1–x_3), and sonication amplitude–extraction time (x_2–x_3)] were found to have no significant effects for both phenolic extraction and AA of date.

Reduced second order regression model for phenolics extraction (Y_{TPC}) and antioxidant activity (Y_{AA}) using significant terms for acetone concentration (x_1), extraction time (x_2), and sonication amplitude (x_3) were given in the following equations:

$$Y_{TPC} = 702.09 + 56.78x_1 - 92.72x_1^2 - 65.77x_2^2 - 79.79x_3^2 \tag{2}$$

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*|
Total phenolic contents (TPC) (mg GAE/100 g DM)				
Intercept	702.09	25.41	27.63	< 0.0001*
x_1	56.78	15.56	3.65	0.01*
x_2	− 33.09	15.56	− 2.13	0.09
x_3	32.54	15.56	2.09	0.09
$x_1 \times x_2$	− 0.17	22.00	− 0.01	0.99
$x_1 \times x_3$	50.62	22.00	2.30	0.07
$x_2 \times x_3$	− 49.48	22.00	− 2.25	0.07
$x_1 \times x_1$	− 92.72	22.90	− 4.05	0.01*
$x_2 \times x_2$	− 65.77	22.90	− 2.87	0.03*
$x_3 \times x_3$	− 79.79	22.90	− 3.48	0.02*
Antioxidant activity (AA) (mg GAE/100 g DM)				
Intercept	39.67	1.61	24.68	< 0.0001*
x_1	3.43	0.98	3.49	0.02*
x_2	− 2.33	0.98	− 2.37	0.06
x_3	3.48	0.98	3.53	0.02*
$x_1 \times x_2$	2.90	1.39	2.09	0.09
$x_1 \times x_3$	1.19	1.39	0.85	0.43
$x_2 \times x_3$	1.18	1.39	0.85	0.44
$x_1 \times x_1$	− 5.44	1.45	− 3.75	0.01*
$x_2 \times x_2$	− 1.08	1.45	− 0.75	0.49
$x_3 \times x_3$	− 3.42	1.45	− 2.36	0.06

x_1, solvent concentration (%); x_2, sonication amplitude (%); x_3, time (min)

*P < 0.05

$$Y_{AA} = 39.67 + 3.43x_1 + 3.48x_3 - 5.44x_1^2 \tag{3}$$

P values for both responses were 0.022 and 0.031, respectively, attested a good model fits. In addition, other parameters are required to ensure the performance of models, which were lack of fit and correlation coefficients. The statistical significance of Eqs. 2 and 3 was checked, and ANOVA results of experimental models were illustrated in Table 3. It indicated that quadratic models were significant (P < 0.05), lack of fits were insignificant (P > 0.05), and correlation coefficients were 0.928 and 0.916 for TPC and AA models, respectively.

Table 3 ANOVA results for the effects of acetone concentration, sonication amplitude, and extraction time on TPC and antioxidant activity

Source	DF[a]	Sum of squares	F ratio	Prob > F
Total phenolic contents (TPC) (mg GAE/100 g DM)				
x_1	1	25,794.02	13.32	0.01*
x_2	1	8760.25	4.52	0.09
x_3	1	8470.16	4.37	0.09
$x_1 \times x_2$	1	0.11	0.00	0.99
$x_1 \times x_3$	1	10,247.51	5.29	0.07
$x_2 \times x_3$	1	9792.09	5.06	0.07
$x_1 \times x_1$	1	31,745.33	16.39	0.01*
$x_2 \times x_2$	1	15,972.40	8.25	0.03*
$x_3 \times x_3$	1	23,504.66	12.14	0.02*
Model	9	125,080.94	7.18	0.022*
Lack of fit	3	8665.64	5.67	0.154
Error	5	9684.19		
Total model	14	134,765.13		
R^2 = 0.928				
Adj. R^2 = 0.799				
Antioxidant activity (AA) (mg GAE/100 g DM)				
x_1	1	94.33	12.17	0.02*
x_2	1	43.57	5.62	0.06
x_3	1	96.61	12.46	0.02*
$x_1 \times x_2$	1	33.70	4.35	0.09
$x_1 \times x_3$	1	5.62	0.72	0.43
$x_2 \times x_3$	1	5.57	0.72	0.44
$x_1 \times x_1$	1	109.08	14.07	0.01*
$x_2 \times x_2$	1	4.31	0.56	0.49
$x_3 \times x_3$	1	43.13	5.57	0.06
Model	9	422.64	6.06	0.031*
Lack of fit	3	32.24	3.30	0.99
Error	5	38.75		
Total model	14	461.40		
R^2 = 0.916				
Adj. R^2 = 0.765				

x_1, solvent concentration (%); x_2, sonication amplitude (%); x_3, time (min)

[a]degrees of freedom

*P < 0.05

Response surface analysis

RSM following Box–Behnken design was applied to determine optimal levels for the three parameters, acetone concentration (x_1), sonication amplitude (x_2), and extraction time (x_3). Surface response plots of models were considered as the best way for illustrating effects of any independent variable and their interactions on responses.

Figure 1A, B showed the effect of acetone concentration and ultrasound amplitude on TPC and AA. The coefficient estimated for quadratic and linear terms of solvent concentration were statistically significant in both responses. Different solvent systems have been used to extract antioxidants from different plant materials because their extraction depends on their chemical nature, polarity, and their state (free or bound to other molecules). In this investigation, acetone was selected as extraction solvent. This choice was consistent with those obtained by Benmeddour et al. [8] and Kchaou et al. [9] for date. According to Alothman et al. [18], acetone/water mixtures are a good solvents for the extraction of polar antioxidants, they were more effective for the dissolution of phenolic compounds. Aqueous acetone solvent could extracted higher amount of phenolics from fruit samples and exhibited higher antioxidant capacity [19].

The results showed that TPC increased with increasing acetone concentration until reaching 66.71% (v/v) which was considered as optimal acetone concentration. The addition of water to acetone caused an increase of polarity, which promotes extraction of relatively less polar components. However, beyond the optimal acetone concentration (66.71%) the responses decreased caused by the inadequacy of solvent polarity [20].

Figure 2A, B illustrated effects of acetone concentration and extraction time on TPC and AA. UAE greatly reduced the extraction time of antioxidants, an extraction during 29.58 min allowed to extract the maximum amount of TPC and exerted the strongest AA. Contrarily, traditional extraction method was laborious and time consuming, extraction of phenolics by maceration during 48 h allowed to extract only 23 mg GAE/100 g of date fruit [10].

The results showed that responses increased with increasing extraction time until 29.58 min. Beyond this time, both TPC and AA responses decreased and this could be explained by degradation of active compounds during prolonged extraction time.

Figure 3A, B illustrated effects of extraction time and sonication amplitude on TPC and AA. Quadratic effect of amplitude was much significant compared to extracting time on TPCs. The rise of sonication amplitude induced increase of agitation, cavity collapse, and thus higher extraction yield [21]. However, during a certain exposure period of extracted antioxidants under high amplitude of sonication amplitude, free radicals were generated which can oxidize phenolic compounds and thus reduce AA [22]. In other hand, high sonication amplitudes can lead to rapid deterioration of the ultrasonic transducer, resulting in liquid agitation instead of cavitation and in poor transmission of the ultrasound through the liquid media [21].

## Determination and validation of optimal conditions

Statistical analysis of results showed the success of the two established models for TPC and AA (correlation coefficient > 0.9, P value for both responses < 0.05, and the lack of
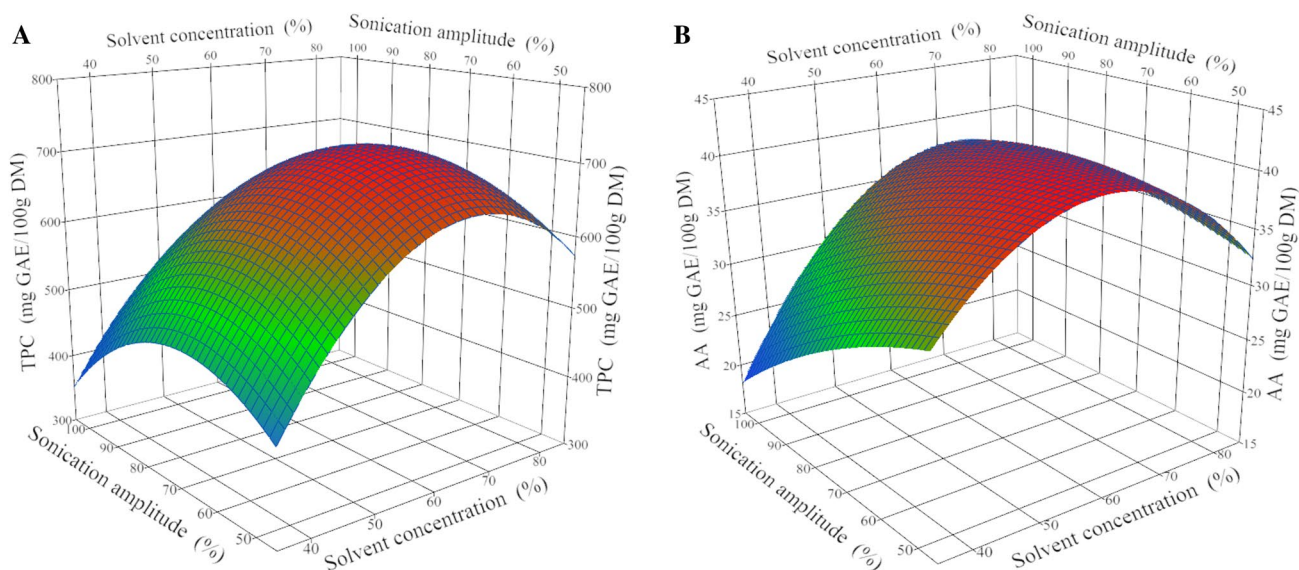
Fig. 1 Response surface plots showing the effects of acetone concentration (%) and ultrasound amplitude (%) on **A** TPC (mg GAE/100 g DM) and **B** antioxidant activity (mg GAE/100 g DM) from date fruit
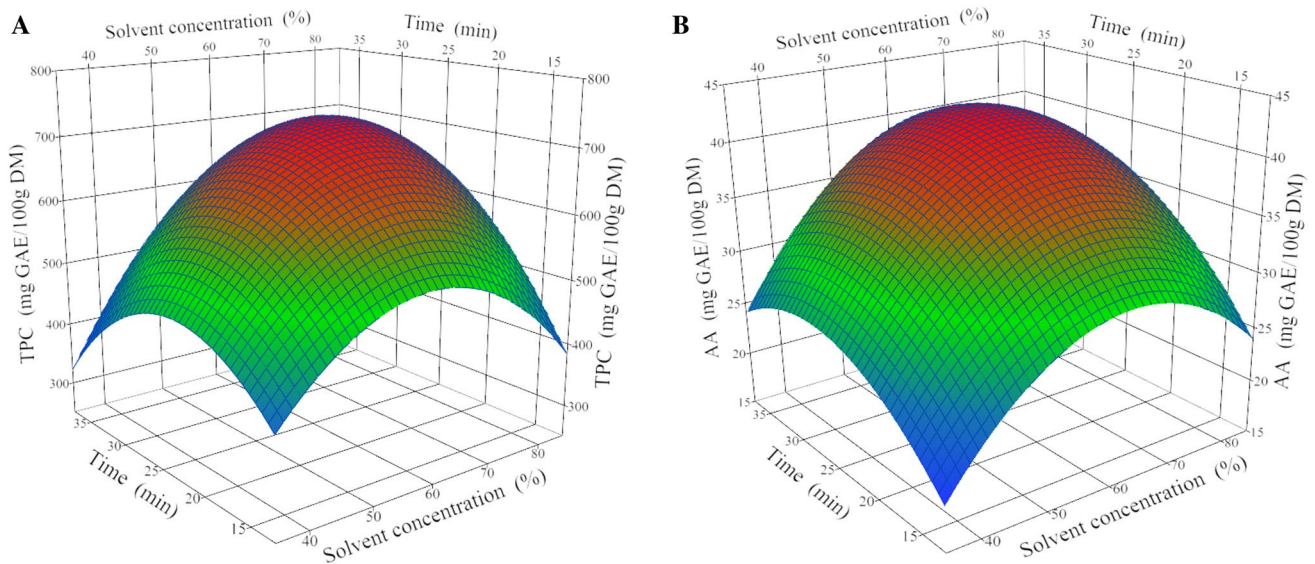
Fig. 2 Response surface plots showing the effects of acetone concentration (%) and time (min) on **A** TPC (mg GAE/100 g DM) and **B** antioxidant activity (mg GAE/100 g DM) from date fruit
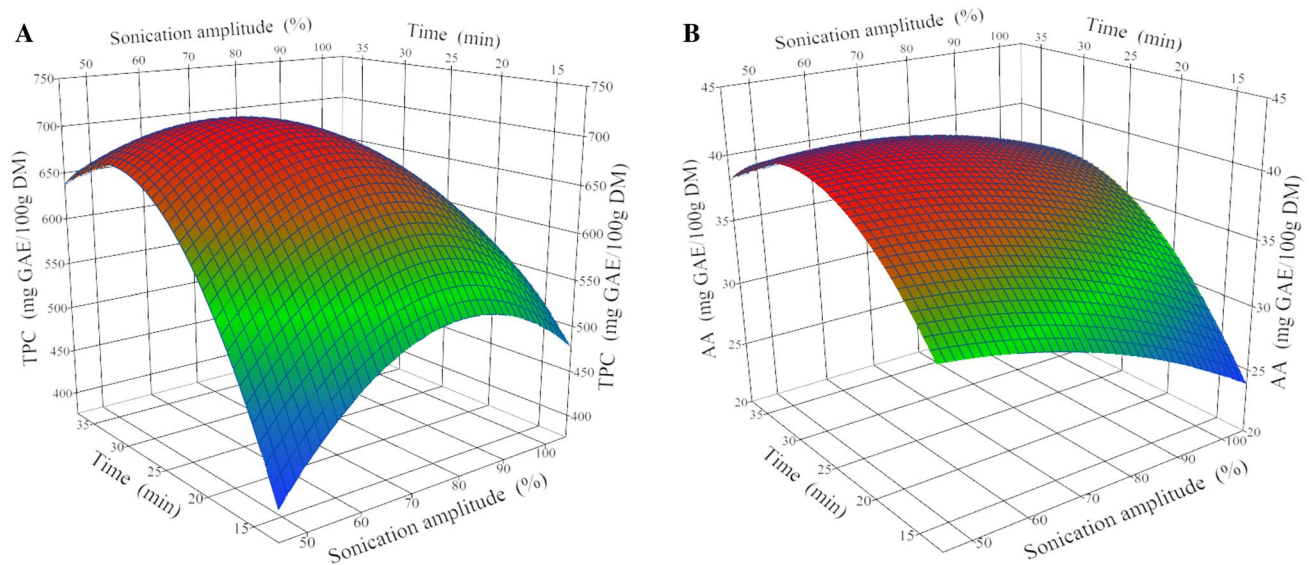
Fig. 3 Response surface plots showing the effects of sonication amplitude (%) and time (min) on **A** TPC (mg GAE/100 g DM) and **B** antioxidant activity (mg GAE/100 g DM) from date fruit

fit were insignificant > 0.05). Nevertheless, the last step to affirm validity of models was to maximize desirability using JMP prediction profiler and compare predicted values with experimental ones. Optimal predicted conditions were acetone concentration of 66.71% under sonication amplitude of 64.78% during an extraction time of 29.58 min. The theoretical predicted values were 728.47 mg GAE/100 g DM for TPC and 41.42 mg GAE/100 g DM for AA. The obtained experimental values were 725.33 ± 1.71 and 39.61 ± 1.06 mg GAE/100 g, respectively. Student t test

indicates that predicted and experimental values were statistically similar ($P < 0.05$). This indicates the validity of the two established models for extraction of TPC and AA.

Conclusion

In the current study, UAE of TPC and AA of date fruit were optimized using RSM. The results indicated that extraction of TPC and AA of date were significantly influenced by

acetone concentration, sonication amplitude, and extraction time. Experimental values were in agreement with predicted ones indicating the suitability of developed quadratic models. Therefore, it was suggested that obtained optimal conditions can be used to extract antioxidant compounds from date palm fruit.

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