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FULL LENGTH ARTICLE

Optimization of antioxidant activity and total polyphenols of dried apricot fruit extracts (*Prunus armeniaca* L.) using response surface methodology

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RSM

Abstract Apricot is a natural source of polyphenols and other phytochemicals such as β-carotene and ascorbic acid that contribute to its antioxidant activity. Various organic solvents such as hexane, ether, methanol, and ethanol are used to obtain fruit extracts for different purposes. However, to extract the vital phytochemicals from a fruit, an efficient solvent along with certain other process parameters could reduce the process inputs thereby increasing the process efficiency. Response surface methodology (RSM) was employed to optimize the conditions for antioxidant potential and polyphenols from apricot powder (*Prunus armeniaca* L.) using four independent variables: methanol (20%, 35%, 50%, 65% and 80%), solvent/sample ratio (10, 15, 20, 25 and 30), temperature (20, 30, 40, 50 and 60 °C) and time (20, 30, 40, 50 and 60 min). The results showed that antioxidant potential and total polyphenols in the experiments varied from 76.15% to 96.68% and 8.77 to 12.11 mg GAE/g, respectively. The *F*-values for antioxidant potential and total polyphenols were 0.99 and 4.44, respectively. The coefficients of determination (R^2 values) for antioxidant potential and total polyphenols were found to be 0.4799 and 0.8057, respectively. Under the optimum conditions of 35% methanol, 15 solvent/sample ratio, 30 °C temperature and time 30 min, the values for antioxidant potential and total polyphenols were 91.165% and 10.702 mg GAE/g, respectively.

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The present process could be employed on a commercial scale for the extraction of antioxidants from apricot fruits for their nutraceutical and other applications.

1. Introduction

Apricot (*Prunus armeniaca* L.) is a hard tree, bearing stone fruit, which is classified under the *Prunus* species of *Prunoidae*, sub family of the *Rosaceae*, family of the Rosales group (Haciseferogullari et al., 2007). *P. armeniaca* is originally from china but arrived in Europe via Armenia, which gave it the scientific name (Bailey and Hough 1975). It is an edible fruit mainly cultivated in Mediterranean climates. Data from FAOSTAT (2013) revealed that >3.8 million tons of apricots were produced in the world in 2011, although a few countries (Turkey, Iran, Italy, Pakistan, France, Spain, USA and Morocco) account for more than 80% of that production. Turkey is the leading country in the production of apricots, rich genetic resources and produces high quality dried apricot cultivars, especially the city of Malatya in Turkey. For this reason, the city of Malatya is also called the apricot capital of the world (Asma 2007). *P. armeniaca* trees can only grow in certain regions of the world where the environmental conditions are favorable. In India, apricot is grown commercially in the hills of Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh and to a limited extent in North Eastern Hills. In Jammu and Kashmir, it is especially grown in Ladakh region.

Functional foods find a very important place in the modern times, where different types of cancer, diabetes, cardiovascular diseases, etc. are increasing day by day (Wani et al., in press). As a functional food, *P. armeniaca* has become a product of interest in recent years because of its nutritional and health benefits. It is a rich source of β-carotene, ascorbic acid, carbohydrates, vitamins, minerals, fiber besides having attractive color and typical flavor (Doymaz 2004; Hussain et al., 2011; Jiménez et al., 2008; Sharma et al., 2012). They are considered to be a rich source of antioxidants that have been reported to overcome some of the degenerative diseases that affect humans. Apricot has been used in folk medicine as a remedy for various diseases. Other uses for apricots in folk medicine include treatment of hemorrhages, infertility, eye inflammation, and spasm. In addition, apricots exhibited anti-carcinogenic, anti-bacterial, anti-viral, anti-tumor and anti-inflammatory properties.

The total phenols and DPPH antioxidant activity of aqueous methanolic extract of apricot can be affected by many factors including methanol, solvent/sample ratio, temperature and extraction time. Under this kind of situation, where multiple variables may influence the output, response surface methodology is an effective technique for optimizing the process (Bas and Boyaci 2007). RSM is exploited for designing statistical experiments, modeling the processes, verifying the statistical significance of the independent variables, and to obtain the optimum operating conditions of the entire process (Sarfarazi et al., 2015; Salimi et al., 2014). The methodology involves three steps: (1) experimental design, in which the independent variables and their experimental levels are set using well-established statistical experimental designs such as the central composite design (CCD); (2) response surface modeling through regression analysis; and (3) process optimization using the response surface models. As a powerful statistical and mathematical tool, RSM has a major advantage over the one factor a time approach in that it allows the evaluation of the effect of multiple variables and their interactions on the output variables with reduced number of trials (Liyana-Pathirana and Shahidi 2005).

The objective of the present work was to optimize the conditions, including methanol, solvent/sample ratio, temperature and extraction time, for antioxidant activity and total phenols from apricot fruit using the response surface methodology.

2. Materials and methods

2.1. Materials

Sun dried apricots (*P. armeniaca* L.) var. Halman of high quality grade from Srinagar, Jammu and Kashmir, were used in this research. The apricots were converted to powder using an electric grinder, packaged in polyethylene bags and kept in a dark place at room temperature. The chemicals used in this research were purchased from HiMedia and were of analytical grade.

2.2. Extraction procedure

Dried apricot powder was heat extracted in a water bath (Major Science, Stirring water bath) using the different combinations of the variables as per the experimental setup provided by the Central Composite Design. The extract was filtered through Wattman No. 4 filter paper and then centrifuged at 10,000 rpm (Eppendorf Centrifuge 5810 R) for 10 min at 4 °C.

2.3. Total polyphenols

Total phenolic content was determined according to Thaipong et al. (2006). 150 μL of extract, 2400 μL of nanopure water and 150 μL of 0.25 N Folin–Ciocalteu reagent were combined and then mixed well using a vortex. The mixture was allowed to react for 3 min then 300 μL of 1 N Sodium carbonate (Na_2CO_3) solution was added and mixed well again by shaking. The solution was incubated at room temperature in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer and the results were expressed as milligram of Gallic acid equivalents (mg GAE) per 1 g of extract using standard curve prepared from Gallic acid solution.

2.4. Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of apricot was determined according to the method of Matthus (2002) as explained in Wani et al. (2013). The

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DPPH radical scavenging assay is regularly used for the relatively rapid evaluation of the antioxidant activity. DPPH is a stable free radical even at room temperature, and shows strong absorbance at 515 nm. The DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule with a different color. Thus the degree of its discoloration from purple to yellow is attributed to the hydrogen donating ability of the added compound, which is indicative of its radical scavenging potential.

Briefly, 80 μL of the sample was mixed with 200 μL of 0.05% DPPH in a total volume of 4 ml methanol and allowed to react in the dark for 30 min. The results were expressed as percent inhibition using the relation

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5. Experimental design and statistical analysis

Design-expert software (Design Expert 9.0.3, Stat-Ease Inc.) was used for the experimental design and statistical analysis of the data. Central Composite Design (CCD) was used to examine the effects of the four variables: Methanol (X_1), Solvent/sample ratio (X_2), Temperature (X_3) and Extraction time (X_4). The coded and decoded independent variables used in the RSM design are listed in Table 1. A total of 30 experimental runs were completed and the antioxidant activity of apricot fruit (% inhibition) and total polyphenols (mg GAE/g) expressed as the dependent variables, was determined (Table 2). These variables were expressed individually as a function of the independent variables. A second-order polynomial equation was used to express antioxidant activity (Y_1) and total polyphenols of apricot fruit (Y_2) as a function of the independent variables as follows:

$$Y = \beta_0 + \sum_{i=1}^{4}\beta_i x_i + \sum_{i=1}^{4}\beta_{ii} x_i^2 + \sum_{i<j}^{4}\beta_{ij} x_i x_j$$

where Y represents the response variables, β_0 is a constant, β_i, β_{ii} and β_{ij} are the linear, quadratic and cross-product coefficients, respectively. X_i and X_j are the levels of the independent variables. Two dimensional surface response contour plots were generated by varying two variables within the experimental range and holding the other two constant at the central point. The test of statistical significance was based on the total error criteria with a confidence level of 95.0%.

Table 1 Coded and decoded levels of independent variables used in the RSM design.

Independent variables	Symbols	Levels				
		−2	−1	0	1	2
Methanol	X_1	20	35	50	65	80
Solvent/sample	X_2	10	15	20	25	30
Temperature	X_3	20	30	40	50	60
Time	X_4	20	30	40	50	60

3. Results and discussion

3.1. Antioxidant activity and total polyphenols

The antioxidant activity (expressed as % inhibition) and total polyphenols (expressed as mg GAE/g) of apricot fruit obtained from the 30 experiments are listed in Table 2. The experimental data were used to calculate the coefficients of the second-order polynomial equation and Table 3 summarizes the regression coefficients obtained. Analysis of variance (ANOVA) showed that the second order polynomial model adequately represented the experimental data. The coefficient of multiple determinations (R^2) for the responses of antioxidant activity and total polyphenols being 0.4799 and 0.8057, respectively. This means that the calculated model was able to explain 47.99% and 80.57% of the results in the case of antioxidant activity and total polyphenols, respectively. For any of the terms in the model, a large regression coefficient and a small p-value would indicate a more significant effect on the respective response variables (Quanhong and Caili 2005). The analysis of variance also showed that there was a non significant lack of fit that further validates the model. The mean antioxidant activity and total polyphenols of 30 selected combinations of the independent variables, varied from 76.15% to 96.68% and 8.77 to 12.11 mg GAE/g, respectively (Table 2). The ethanolic antioxidant activity and total polyphenols of Halman apricot were 46.6% and 354.74 mg GAE/100 g, respectively (Hussain et al., 2013). The higher antioxidant activity and total polyphenols in our case could be a combined effect of methanolic solvent and other process parameter. The regression equations obtained for the independent and dependent variables for antioxidant activity (Y_1) and total polyphenols (Y_2) were:

$$Y_1 = 85.02 - 1.01X_1 - 3.02X_2 - 0.24X_3 - 0.48X_4 + 2.08X_1^2 + 1.93X_2^2 + 0.046X_3^2 + 0.24X_4^2 - 1.51X_1X_2 - 0.41X_1X_3 + 0.67X_1X_4 - 1.27X_2X_3 - 0.24X_2X_4 - 0.15X_3X_4$$

$$Y_2 = 9.58 + 0.31X_1 - 0.27X_2 + 0.45X_3 - 0.13X_4 + 0.59X_1^2 + 0.53X_2^2 + 0.23X_3^2 + 0.12X_4^2 - 0.081X_1X_2 - 0.15X_1X_3 + 0.076X_1X_4 + 0.065X_2X_3 + 0.064X_2X_4 + 0.046X_3X_4$$

3.2. Analysis of response surfaces

The best way to visualize the effect of the independent variables on the dependent variables is to draw response surface plots of the model, which were done by varying two variables within the experimental range and holding the other two constant at the central point. Fig. 1a is a response surface plot showing the effect of methanol and solvent/sample ratio on the antioxidant activity of apricot fruit while keeping the time and temperature at the central values of 40 °C and 40 min, respectively. Under the mentioned conditions, the maximum antioxidant activity could be obtained with a methanolic concentration of 40% having a solvent/sample ratio of 15. Methanol as a solvent for various fruit and vegetable extracts exhibited the highest DPPH free radical scavenging activity as compared to acetone and ethanol (Sulaiman et al., 2011). Fig. 1b shows the effect of methanol and temperature on the

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Table 2 Experimental design for antioxidant potential and total polyphenols.

Run	Methanol (%)	Solvent/ sample (v:w)	Temperature (°C)	Extraction time (min)	Experimental antioxidant activity (% inhibition)	Predicted antioxidant activity	Experimental total polyphenols (mg GAE/g)	Predicted total polyphenols
	(X_1)	(X_2)	(X_3)	(X_4)	(EY_1)	(PY_1)	(EY_2)	(PY_2)
1	−1 (35)	−1 (15)	−1 (30)	−1 (30)	94.38 ± 2.06	91.16	10.73 ± 1.11	10.70
2	1 (65)	−1 (15)	−1 (30)	−1 (30)	94.22 ± 2.02	91.63	11.90 ± 1.33	11.64
3	−1 (35)	1 (25)	−1 (30)	−1 (30)	94.63 ± 2.10	91.14	10.07 ± 1.04	10.07
4	1 (65)	1 (25)	−1 (30)	−1 (30)	83.49 ± 2.70	85.59	10.97 ± 1.20	10.69
5	−1 (35)	−1 (15)	1 (50)	−1 (30)	93.56 ± 2.01	94.35	11.30 ± 1.33	11.69
6	1 (65)	−1 (15)	1 (50)	−1 (30)	93.48 ± 2.08	95.16	11.98 ± 1.39	12.02
7	−1 (35)	1 (25)	1 (50)	−1 (30)	87.38 ± 1.79	89.25	10.98 ± 1.10	11.32
8	1 (65)	1 (25)	1 (50)	−1 (30)	79.72 ± 2.65	82.04	11.52 ± 1.20	11.32
9	−1 (35)	−1 (15)	−1 (30)	1 (50)	94.14 ± 2.10	89.63	10.11 ± 1.03	10.07
10	1 (65)	−1 (15)	−1 (30)	1 (50)	95.41 ± 2.07	92.78	11.96 ± 1.39	11.32
11	−1 (35)	1 (25)	−1 (30)	1 (50)	89.10 ± 1.82	88.67	10.03 ± 1.01	9.70
12	1 (65)	1 (25)	−1 (30)	1 (50)	88.77 ± 2.80	91.42	11.25 ± 1.23	10.70
13	−1 (35)	−1 (15)	1 (50)	1 (50)	95.08 ± 1.20	92.23	11.27 ± 1.27	11.25
14	1 (65)	−1 (15)	1 (50)	1 (50)	92.42 ± 1.02	93.71	12.11 ± 1.43	11.88
15	−1 (35)	1 (25)	1 (50)	1 (50)	85.78 ± 0.77	86.18	11.12 ± 1.09	11.14
16	1 (65)	1 (25)	1 (50)	1 (50)	79.18 ± 1.69	81.66	11.71 ± 1.49	11.44
17	−2 (20)	0 (20)	0 (40)	0 (40)	91.11 ± 2.01	95.36	11.74 ± 1.37	11.31
18	2 (80)	0 (20)	0 (40)	0 (40)	92.62 ± 1.05	91.30	11.58 ± 1.39	12.56
19	0 (50)	−2 (10)	0 (40)	0 (40)	93.24 ± 2.12	98.78	12.11 ± 1.48	12.24
20	0 (50)	2 (30)	0 (40)	0 (40)	89.30 ± 2.87	86.69	10.78 ± 1.05	11.18
21	0 (50)	0 (20)	−2 (20)	0 (40)	78.28 ± 2.71	85.68	8.77 ± 0.77	9.61
22	0 (50)	0 (20)	2 (60)	0 (40)	89.18 ± 2.87	84.72	11.73 ± 1.38	11.43
23	0 (50)	0 (20)	0 (40)	−2 (20)	87.13 ± 0.78	86.93	10.58 ± 1.22	10.31
24	0 (50)	0 (20)	0 (40)	2 (60)	81.89 ± 2.76	85.02	8.99 ± 0.89	9.80
25	0 (50)	0 (20)	0 (40)	0 (40)	76.56 ± 2.64	85.01	9.05 ± 0.95	9.58
26	0 (50)	0 (20)	0 (40)	0 (40)	89.02 ± 2.90	85.01	10.40 ± 1.11	9.58
27	0 (50)	0 (20)	0 (40)	0 (40)	89.55 ± 2.93	85.01	9.97 ± 0.91	9.58
28	0 (50)	0 (20)	0 (40)	0 (40)	76.15 ± 0.63	85.01	8.89 ± 0.81	9.58
29	0 (50)	0 (20)	0 (40)	0 (40)	82.13 ± 0.75	85.01	9.24 ± 0.95	9.58
30	0 (50)	0 (20)	0 (40)	0 (40)	96.68 ± 2.25	85.01	9.92 ± 0.98	9.58

Table 3 Analysis of variance (ANOVA) for surface quadratic model.

Responses	Source of variation	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-Value
Antioxidant activity	Model	524.31	14	37.45	0.99	0.5061
	Residual	568.24	15	37.88		
	Lack of fit	237.38	10	23.74	0.36	0.9212
	Pure error	330.86	5	66.17		
	Corr total	1092.55	29			
R^2 = 0.4799 R^2 (adjusted) = 0.4799						
Total polyphenols	Model	25.53	14	1.82	4.44	0.0034
	Residual	6.16	15	0.41		
	Lack of fit	4.36	10	0.44	1.21	0.4422
	Pure error	1.80	5	0.36		
	Corr total	31.69	29			
R^2 = 0.8057 R^2 (adjusted) = 0.6243						

antioxidant activity of apricot fruit while keeping the time and solvent/sample ratio at the central values of 20 and 40 min, respectively. Under these conditions, the maximum antioxidant activity could be obtained with a methanolic concentration of 35% at a temperature of 45 °C. Fig. 1c shows the effect of methanol and extraction time on the antioxidant activity of apricot fruit while keeping the solvent/sample ratio and temperature at the central values of 20 and 40 °C, respectively. Under the said conditions, the maximum antioxidant activity could be obtained with a methanolic concentration of 35% for the extraction time of 30 min. Fig. 1d shows the effect of solvent/sample ratio and temperature on the antioxidant activity of apricot fruit while keeping methanol and extraction time at the central values of 50% and 40 °C, respectively. Under these conditions, the maximum antioxidant activity could be obtained with a solvent/sample ratio

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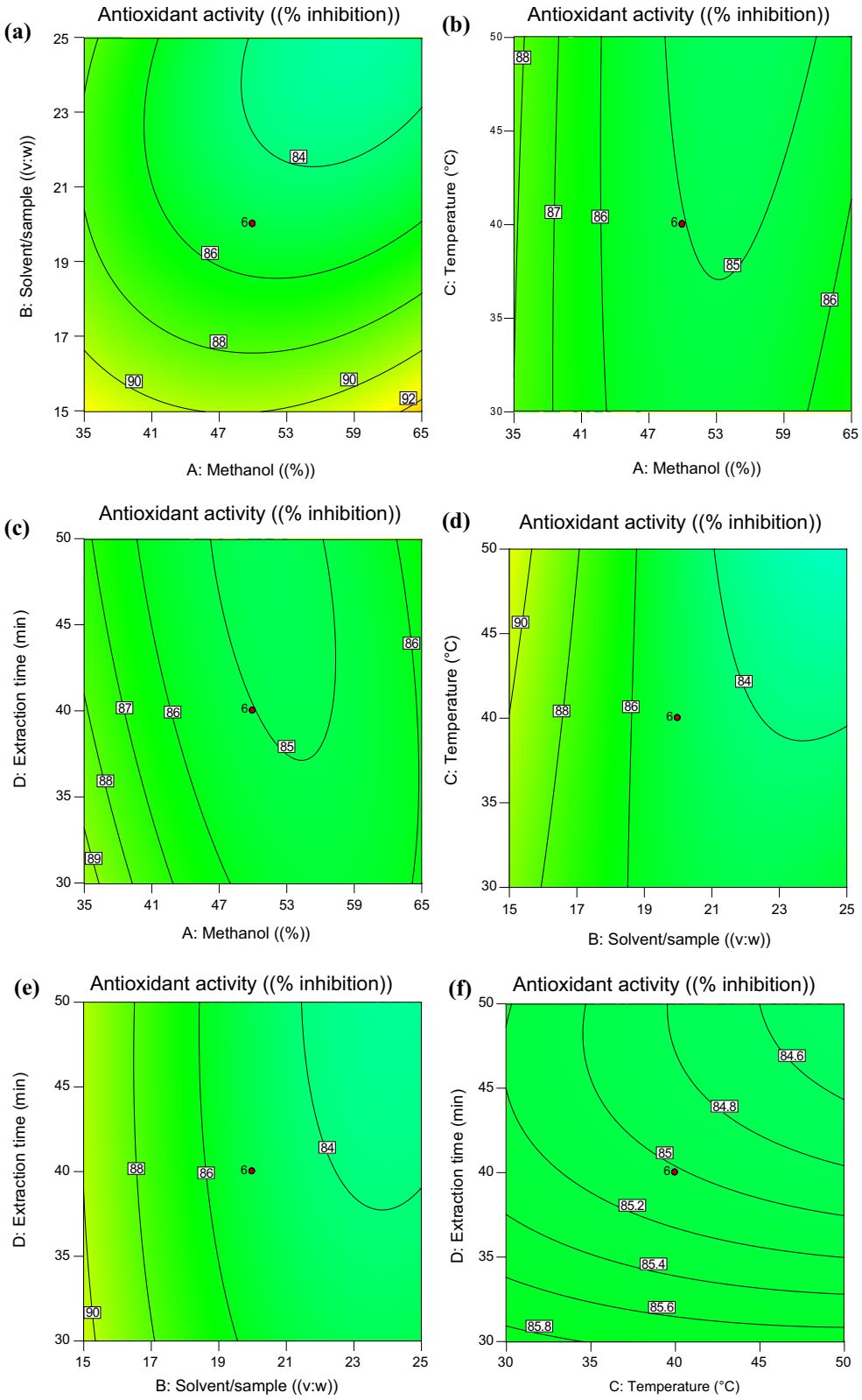

Figure 1 Effect of (a) solvent sample ratio and methanol, (b) temperature and methanol, (c) extraction time and methanol, (d) temperature and solvent sample ratio, (e) extraction time and solvent sample ratio and (f) extraction time and temperature on antioxidant potential of apricot fruit, keeping the other two variables at their respective central values in each case.

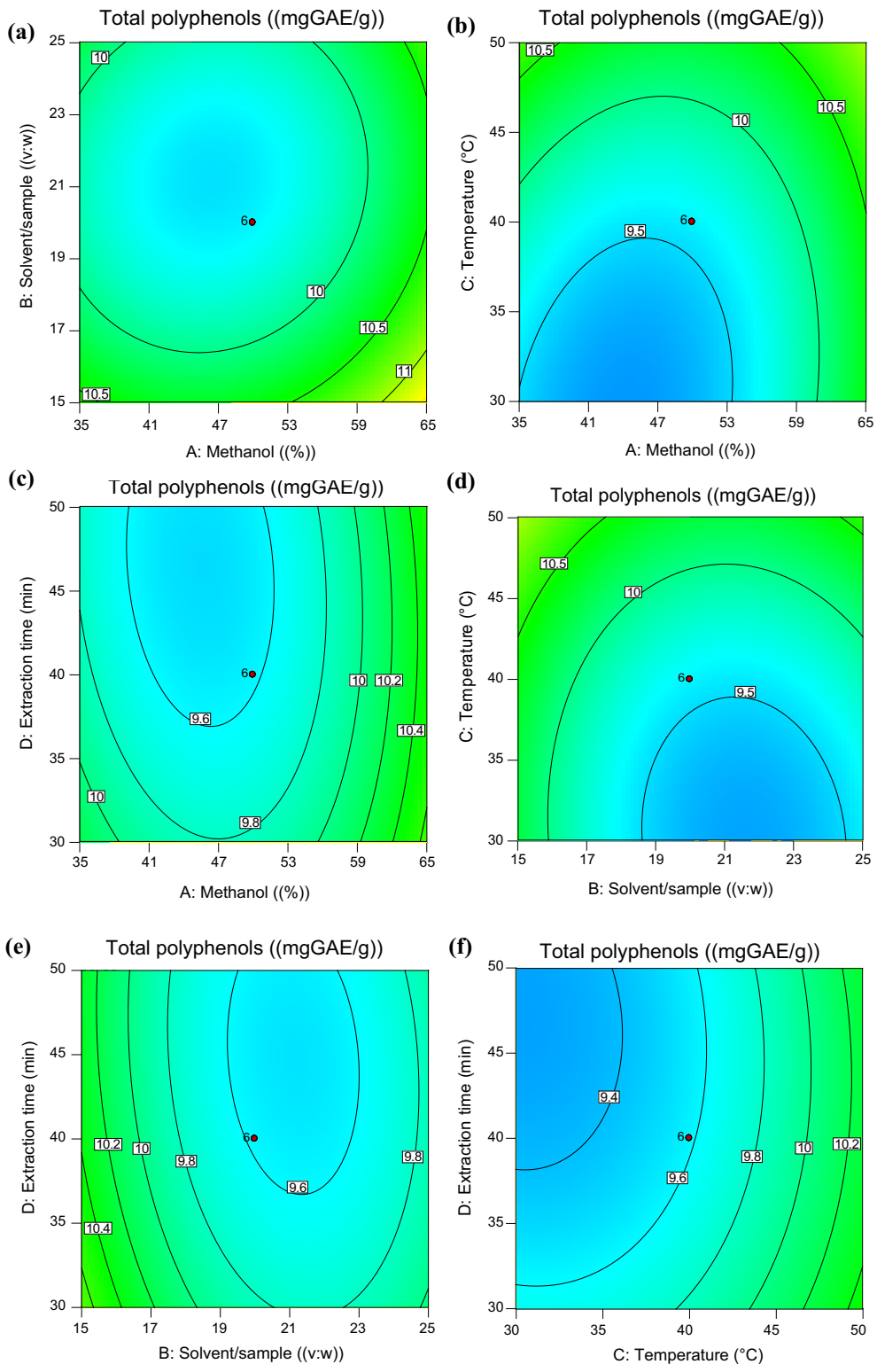

Figure 2 Effect of (a) solvent sample ratio and methanol, (b) temperature and methanol, (c) extraction time and methanol, (d) temperature and solvent sample ratio, (e) extraction time and solvent sample ratio and (f) extraction time and temperature on total polyphenols of apricot fruit, keeping the other two variables at their respective central values in each case.

of 15, at a temperature of 45 °C. Fig. 1e shows the effect of solvent/sample ratio and extraction time on the antioxidant activity of apricot fruit while keeping methanol and temperature at the central values of 50% and 40 °C, respectively. Under these conditions, the maximum antioxidant activity could be obtained with a solvent/sample ratio of 15

for a time of 33 min. Fig. 1f shows the effect of temperature and extraction time on the antioxidant activity of apricot fruit while keeping methanol and solvent/sample ratio at the central values of 50% and 20%, respectively. Under these conditions, the maximum antioxidant activity could be obtained at a temperature of 30 °C and extraction time of 30 min.

Fig. 2a shows the effect of methanol and solvent/sample ratio on the total polyphenolic content of apricot fruit while keeping extraction time and temperature at the central values of 40 min and 40 °C, respectively. Under these conditions, the maximum total polyphenolic content could be obtained with 55% methanol and a sample solvent ratio of 63. Fig. 2b shows the effect of methanol and temperature on the total polyphenolic content of apricot fruit while keeping extraction time and solvent/sample ratio at the central values of 40 and 20 min, respectively. Under these conditions, the maximum total polyphenolic content could be obtained with 62% methanol at a temperature of 46 °C. Fig. 2c shows the effect of methanol and extraction time on the total polyphenolic content of apricot fruit, while keeping temperature and solvent/sample ratio at the central values of 40 and 20 °C, respectively. Under these conditions, the maximum total polyphenolic content could be obtained with 65% methanol at a temperature of 40 °C. Fig. 2d shows the effect of solvent/sample ratio and temperature on the total polyphenolic content of apricot fruit while keeping methanol and extraction time at the central values of 50% and 40 min, respectively. Under these conditions, the maximum total polyphenolic content could be obtained with a solvent/sample ratio of 16 and at a temperature of 47 °C. Fig. 2e shows the effect of solvent/sample ratio and extraction time on the total polyphenolic content of apricot fruit while keeping temperature and methanol at the central values of 40 °C and 50%, respectively. Under these conditions, the maximum total polyphenolic content could be obtained with a solvent/sample ratio of 15, and extraction time of 30 min. Fig. 2f shows the effect of temperature and extraction time on the total polyphenolic content of apricot fruit while keeping solvent/sample ratio and methanol at the central values of 20% and 50%, respectively. Under these conditions, the maximum antioxidant activity could be obtained with at a temperature of 50 °C for the extraction time of 30 min. The total polyphenolic content obtained in our experiments showed higher values than the previous reports (Ouchemoukh et al., 2012; Gulcin et al., 2010; Gomaa 2013). Using a combination of 80% methanol and a sample to solution ratio of 1:2, a total polyphenolic content of 354.74 mg GAE/100 g was obtained from Halman apricots (Hussain et al., 2013). The reasons for the higher total phenols in our experiments could be the combined effect of solvent, time of extraction, temperature and solvent/sample ratio.

3.3. Process optimization and confirmatory study

Using a criterion of desirability that included the highest antioxidant activity and total polyphenolic content, while keeping minimum solvent percentage, solvent/sample ratio, time and temperature, it was possible to optimize the extraction process. The optimum extraction conditions for the present study were aqueous methanol of 35%, solvent/sample ratio of 15, temperature of 30 °C and extraction time of 30 min, at which the values for antioxidant potential and total polyphenols were 91.16% and 10.70 mg GAE/g, respectively. The validity of the design can be determined by the fact that the optimum processing conditions are a combination of the central composite design followed for the present analysis. The experimental values for the same process conditions include antioxidant potential and total polyphenolic content values of 94.38 ± 2.06% and 10.73 ± 1.11 mg GAE/g, respectively. The experimental and predicted values were within the range and did not vary significantly at 5% level. Therefore, the regression equations obtained in this study can be used to draw extract from apricots with optimum antioxidant activity and total polyphenols.

4. Conclusion

The methanolic extract of apricots was analyzed for its antioxidant potential and the corresponding total polyphenols following 30 different combinations of four independent variables, viz. methanol, solvent/sample ratio, temperature and time as per the experimental design. The second-order model developed for apricot extract exhibited a non-significant ($p < 0.05$) value for lack of fit and a low and high value for R^2 for antioxidant potential (0.4799) and total polyphenols (0.8057), respectively. The antioxidant potential and total polyphenols in apricots can be brought to the desired level by different combinations using methanol, solvent/sample ratio, time and temperature. Using Response Surface Methodology, the optimum condition of methanol, solvent/sample ratio, time and temperature was obtained. These optimum conditions were aqueous methanol of 35%, solvent/sample ratio of 15, temperature of 30 °C and extraction time of 30 min, at which the values for antioxidant potential and total polyphenols were 91.16% and 10.702 mg GAE/g respectively. The optimum processing conditions were a combination of the central composite design followed for the present analysis that shows the validity of the experimental design.

Conflict of interest

There is no conflict of interest.

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