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Optimization of Anthocyanin Extraction from Saffron Petals with Response Surface Methodology

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Abstract Optimum extraction conditions of anthocyanins from petals of saffron (*Crocus sativus*) using acidified ethanol as the solvent were revealed. The investigated factors were solvent to sample ratio (20:1–80:1), ethanol concentration (%), extraction temperature (25–45 °C), and time (8–24 h). Response surface methodology with Box–Behnken design was applied to determine optimum processing conditions leading to maximum extraction efficiency (mg cyanindin-3-glucoside/l). Obtained coefficients of variance showed that the linear effect of temperature was more pronounced for extraction yield than three other variables at 5 % level. Optimum extraction conditions that maximize the extracted anthocyanins were found to be a ratio of solvents to sample 20 ml/g, ethanol concentration of 25.02 %, temperature 25.8 °C, and extraction time 24 h which gave 1609.11 mg/l anthocyanins. A quadratic regression equation describing the effects of independent process variables on anthocyanin extraction from saffron petals can be used for finding optimum conditions to achieve desired extraction yield in similar conditions.

Keywords Saffron petal · Conventional extraction · Anthocyanin · Response surface methodology

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Introduction

These days, agricultural by-products removed from food production lines that were considered as useless materials and produced big and complicated problems for the environments in the past are now mostly known as functional foods due to the necessity of use of natural resources in food industries (Galanakis 2012, 2013; Galanakis et al. 2015). Most of the synthetic colors that are used in the food industry have chemical sources with harmful health effects. Since the anticancer and antivirus properties of natural colorants are proven, today there are more tendencies to use natural colorants instead of synthetic ones (Andersen and Jordheim 2010).

Anthocyanins as natural pigments are found in roots, leaves, fruits, and flowers of plants (Chen 2008). Attractive color and functional properties (like prevention of neuronal and cardiovascular, cancer, and diabetes illnesses) of anthocyanins make them a suitable substitute for synthetic pigments in the food industry (Castaoveda-Ovando et al. 2009). There are different sources of anthocyanins like grapes, berries, red cabbage, apples (Castaoveda-Ovando et al. 2009), red potatoes, and black carrots (Kirca et al. 2006). Saffron (*Crocus Sativus*) which produces largely in Iran (Esfanjani et al. 2015). with a share of annually more than 90 % of total saffron in the world (Rajabi et al. 2015). has cyanic color flowers with major colorant of anthocyanins (Norbark et al. 2002). Saffron petals consist a large portion of saffron flower total weight (Hemmati Kakhki et al. 2001). then, these petals could be a big by-product of agriculture and a good source of natural anthocyanins. Many studies have investigated the biomedical and cancer therapy properties of saffron's petal (Kubo and Kinst-Hori 1999; Hadizadeh et al. 2003; Lee et al. 2008; Fatehi et al. 2003; Moshiri et al. 2006; Akhondzadeh Basti et al. 2007; Agha-Hosseini et al. 2008; Khalili et al. 2010). In these studies, it has been reported that the major antioxidant

compounds of petals such as flavonoids, anthocyanins, and flanonons are responsible for these functional properties. Since nearly 86.4 % (wb) or 96.36 % (db) of total weight of saffron flowers belong to their petals (Hemmati Kakhki et al. 2001) and large scale of saffron flowers is released in nature in Iran after picking stigmas annually, potentially, anthocyanins in saffron's petal extract can be used as a natural resource of colorants in food products, adding to its other medicinal/ industrial applications (Kafi 2006).

Generally, extraction of anthocyanins takes place at low temperatures (below 30 °C), preferably under vacuum (to minimize degradation) and in an acidic environment. Solvents like ethanol, methanol, N-butanol, cold acetone, propylene glycol, mixture of methanol—acetone and water, or boiled water can be used in extraction of anthocyanins. Among the various solvents, ethanol is usually preferred for its low toxicity. Use of mineral acids such as hydrochloric acid preserves this pigment through extraction in its stable form (ion flavylium) by reducing pH of solvents) Gould et al. 2008 .(In a study by Kirca et al (2007) which was carried out on the anthocyanin extraction from black carrot, it was observed that rising extract pH from 4.3 to 6 destroyed anthocyanins, and generally, in solutions with pH > 5, monomeric anthocyanins significantly ($P < 0.05$) decreased. Hydrochloric acid may cause deformation of anthocyanins including hydrolysis in acidic conditions during evaporation in a rotary evaporator (40 °C))Van Sumere et al. 1985)or deacylation of acylated anthocyanins with aliphatic acids which can occur at room temperature)Harbourne et al. 2013.(Acetylated anthocyanins are more stable than their non-acetylated analogues (Yoshida et al. 1991.(

Temperature is another factor in conventional solvent extraction of anthocyanins. According to Einstein's equation, $\left(D \propto \frac{T}{\eta}\right)$ rising temperature increases diffusion coefficient, and thus, ingredients can be extracted faster. Furthermore, denaturation of plant cell walls which occurs at high temperatures makes removal of anthocyanins from plant tissues easier (Cacace and Mazza 2003a). However, the vulnerability of anthocyanins to heat and deterioration of these pigments into brown or colorless polymeric pigments and disappearance of desired color of extract exposed to heat treatment cannot be easily ignored (Pala and Toklucu 2012; Patras et al. 2010; Stintzing and Carle 2004). One solution could be encapsulation of anthocyanins by natural biopolymers (Akhavan et al. 2014; Khazaei et al. 2014).

Response surface methodology (RSM) is as an approach to build approximated models based on data collected during physical examination, simulated by computer and experimented observations (Sarfarazi et al. 2015). In RSM, a set of mathematical-statistical models are used for engineering and modeling procedures. The main purpose of this technique is to optimize the response surface that is influenced by factors of the process (Raissi and Eslami Farsiani 2009).

In this study, four conventional extraction parameters in saffron petal's extraction including ethanol percent, ratio of solvent to sample, extraction time, and temperature were optimized by RSM, in order to achieve maximum extraction yield of anthocyanins.

Materials and Methods

Samples and Chemicals

Saffron flowers were collected before sunlight from a farm near Torbat-E-Heydariyeh (Iran) on November 2013. After removing stigmas and anther, the petals were dried in a dark and warm room (37 °C) in front of a fan. This method of drying was compared with three other methods of drying in oven, 70 °C for 6 h, vacuum oven drying, 40 °C for 24 h, and conventional drying, 25 °C for 3 days, and mentioned that method was chosen due to the most stability of anthocyanins through drying. Dried petals were crushed and sieved (16 meshes) and were kept in airtight bags in around 5 °C. Analytical grade hydrochloric acid (HCL) and ethanol were purchased from Merck (Darmstadt, Germany). Distilled water was used for preparation of all solutions. Potassium chloride buffer pH 1.0 and sodium acetate buffer pH 4.5 used in this study were of analytical grade.

Saffron Petal Analysis

Some physicochemical properties of the saffron petal were analyzed including moisture content, proteins, total ash, total fiber and total sugar, lipids (AOAC 2006), and total monomeric anthocyanin content (Lee et al. 2008).

Extraction of Anthocyanins from Saffron Petals

In order to extract anthocyanins, the dried petals were added to 30 ml of acidic ethanol (pH = 2). Amount of petals, extraction time, temperature, and ethanol percentage which were selected based on RSM experimental design are presented in Table 1. Experiments were done in brown color bottles with screwed caps. After extraction, samples were filtered through filter paper (Whatman No. 1). Total anthocyanins of extracts were measured with pH differential method which was adopted from Giusti and Wrolstad (2001). In this method, the extracts were added to buffers 1.0 and 4.5 and allowed to equilibrate for 20 min. The absorbance of each equilibrated solution was then measured at 520 nm (λ max) and 700 nm for haze correction, using an UV–Vis spectrophotometer (Shimatzu-160A

Table 1 Total anthocyanin content of saffron petal's extract through conventional extraction in Box-Behnken design

No.	Independent variables				Total anthocyanin content
	X_1 Time (h)	X_2 Temperature (°C)	X_3 Ethanol percent (%)	X_4 Solvent ratio (ml/g)	mg/l extract
1	8 (−1)[a]	45 (1)	50 (0)	50 (0)	1248.24
2	8 (−1)	35 (0)	50 (0)	20 (−1)	1050.01
3	8 (−1)	25 (−1)	50 (0)	50 (0)	1511.25
4	8 (−1)	35 (0)	25 (−1)	50 (0)	1214.46
5	8 (−1)	35 (0)	75 (1)	50 (0)	1419.4
6	8 (−1)	35 (0)	50 (0)	80 (1)	1495.46
7	8 (−1)	35 (0)	50 (0)	50 (0)	1190.54
8	16 (0)	45 (1)	50 (0)	20 (−1)	1011.95
9	16 (0)	35 (0)	50 (0)	50 (0)	1202.03
10	16 (0)	45 (1)	75 (1)	50 (0)	993.67
11	16 (0)	25 (−1)	50 (0)	20 (−1)	1110.24
12	16 (0)	35 (0)	50 (0)	50 (0)	1200.95
13	16 (0)	25 (−1)	75 (1)	50 (0)	1287.29
14	16 (0)	45 (1)	25 (−1)	50 (0)	1178.47
15	16 (0)	35 (0)	50 (0)	50 (0)	1196.04
16	16 (0)	45 (1)	50 (0)	80 (1)	1035.02
17	16 (0)	35 (0)	75 (1)	80 (1)	1163.94
18	16 (0)	25 (−1)	50 (0)	80 (1)	1436.1
19	16 (0)	35 (0)	50 (0)	50 (0)	1201.36
20	16 (0)	35 (0)	75 (1)	20 (−1)	852.54
21	16 (0)	35 (0)	25 (−1)	20 (−1)	1210.54
22	16 (0)	25 (−1)	25 (−1)	50 (0)	1340.92
23	16 (0)	35 (0)	25 (−1)	80 (1)	1157.2
24	24 (1)	25 (−1)	50 (0)	50 (0)	1275.09
25	24 (1)	35 (0)	75 (1)	50 (0)	962.65
26	24 (1)	45 (1)	50 (0)	50 (0)	1042.23
27	24 (1)	35 (0)	25 (−1)	50 (0)	1446.23
28	24 (1)	35 (0)	50 (0)	80 (1)	1175.27
29	24 (1)	35 (0)	50 (0)	20 (−1)	1300.14

[a] Numbers in parenthesis show independent variables in coded units

,Japan). Pigment content in acidic ethanol was calculated based on cyanidin-3-glucoside (Lee et al 2008). The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{\text{vis-max}} - A_{700})_{\text{pH 1.0}} - (A_{\text{vis-max}} - A_{700})_{\text{pH 4.5}} \quad (1)$$

The total anthocyanin content in the original sample was calculated using the following formula:

$$\text{Total anthocyanin content}(\text{mg/l}) = (A \times \text{MW} \times \text{DF} \times 1000)/(\alpha \times L) \quad (2)$$

where MW is the molecular weight of delphinidin. Since major anthocyanin content of saffron petal was not clear, we used delphinidin MW (Giusti and Wrolstad 2001). DF is the dilution factor, and α is the molar absorptivity.

Total Pigment Content of Saffron's Petal

Total pigment content of petals was measured base on the method by Hemmati Kakhki et al. (2001) with some modifications. A total of 5 ± 0.01 g powdered sample was mixed with 200 ml HCL-50 % ethanol (pH = 2). Extraction temperature was set at 25 ± 1 °C. Experiment was carried out for 8 h while it was mixing slowly (100 rpm). Extract was filtered through Whatman No. 1 paper under vacuum and collected in a volumetric flask. The residue was taken back and extracted again in the same conditions. The anthocyanin contents of double extractions were mixed and used for determination of the total anthocyanins.

Statistical Analysis

In order to estimate effect of independent variables on total anthocyanin content, response surface methodology was applied using a commercial package, Design-Expert version 8.0.1 (Statease Inc., Minneapolis, USA). Box-Behnken design was used, and the design consisted of 29 experiments shown in Table 1 with five replicates in center point and two replicates in every experiment. Statistical significance of the terms in the regression equation was examined. Response surface plots were generated with the same software. Optimum levels of independent variables for the procedure (solvent ratio, extraction time, ethanol percent, and temperature) were determined through optimization by RSM.

Results and Discussion

Physicochemical Characteristics of Saffron's Petal

In Table 2, some physicochemical properties of the saffron's petal are represented. Total anthocyanin content of extract was equal to 1712.19 ± 60 mg delphinidin/l. Lee et al (2008) in their investigation reported that total anthocyanin contents in some natural resources based on mg cyanidin-3-glocoside/l were as cranberry juice, 13.6; red wine, 201.6; natural colorant, 640.8; raspberry juice, 336.7; and elderberry 3006.8 mg/l. Total anthocyanin content in black carrot was determined as 1750 mg/kg (wb) by Kirca et al. (2007). As it can be seen, the saffron petal in comparison with others is a good natural source of anthocyanin with high capacity.

Extraction of Total Anthocyanin Content from Saffron Petal

Obtained total anthocyanin content through conventional solvent extraction based on mg delphinidin/l (per liter of extract) is represented in Table 1. After analysis of variance and based on F and P values, a second-order polynomial response surface model (Eq. (3) and Table 3) was fitted to the response variable (Y). X_1, X_2, X_3, and X_4 were independent variables, namely, solvent ratio, ethanol percent, temperature, and time, respectively. Based on ANOVA test (Table 4), four studied variables had a significant ($P<0.01$) linear effect on the model. Quadratic effect of solvent ratio and temperature (x_1^2, x_4^2; $P<0.01$) and the interaction effects of all independent variables except interaction of ethanol percent and temperature (X_2*X_3) and solvent and ethanol percent (X_1*X_2), with 99 % confidence, were significant.

$$\begin{aligned}\text{Anthocyanin} = {} & 1198.18-61.44x_1-120.19x_2-73.11x_3 \\ & +77.31x_4\ -172.\ 13\,x_1x_3-142.61x_1x_4 \\ & -75.7x_2x_4+91.18x_3x_4+88.92x_1^2 \\ & -53.84\,x_4^2\end{aligned} \quad (3)$$

Represented model (Eq. (3)) is the second-order polynomial equation (regression model) after omitting insignificant coefficients in 99 % confidence level. Constant number (1198.18) is constant coefficient of polynomial model. Y is total anthocyanin content (mg/l) based on delphinidin. As it can be seen, among four independent variables, only the solvent had a positive effect and other three independent variables had negative effects on total anthocyanin content in experimental conditions. Also among mentioned factors, time and temperature had highest and lowest effects on response, respectively. Coefficient of determination, R^2, was 0.948, and adjusted R^2 was 0.92. When R^2 is near to unity, it means that fitted empirical model is suitable for actual data and the model can explain the relation among variables (Koocheki et al. 2009). In Fig. 1, the model predicted and actual values of anthocyanins obtained during the extraction have been compared.

Response Surface Plots of Extraction Efficiency

The interaction of time and temperature on total anthocyanin content is shown in Fig. 2a. In all response surface plots, two other variables which are not mentioned are in their midpoints. During all extraction times, increasing the temperature reduced the total anthocyanin content linearly. Monomeric

Table 2 Physicochemical properties of saffron petals

Properties	Results	Unit	Method
Protein	8.73 ± 0.09	g/100 g dry matter	AOAC (2006)
Lipid	10.25 ± 0.09	g/100 g dry matter	AOAC (2006)
Fiber	11.39 ± 0.11	g/100 g dry matter	AOAC (2006)
Total sugar	13.05 ± 0.12	g/100 g dry matter	AOAC (2006)
Total ash	5.76 ± 0.003	g/100 g dry matter	AOAC (2006)
Moisture	87.11 ± 0.04	g/100 g dry matter	AOAC (2006)
Total anthocyanins	1712.19 ± 60	mg/l extract	(Lee et al. 2008)

Table 3 Regression coefficients for total anthocyanin content of saffron petal's extract in quadratic model with conventional solvent extraction

Term	Coefficient	Standard error for the coefficient	*F*	*P* value
Intercept	1198.18	20.07	25.05	<0.0001
X_1 time	−61.44	12.96	22.49	<0.0003
X_2 temprature	−120.19	12.96	86.07	<0.0001
X_3 ethanol (%)	−73.11	12.96	31.85	<0.0001
X_4 solvent ratio	77.31	12.96	35.61	<0.0001
x_1^2	88.92	17.62	25.46	<0.0002
x_2^2	4.03	17.62	0.052	0.8223
x_3^2	−26.36	17.62	2.24	0.1568
x_4^2	−53.84	17.62	0.33	0.0086
X_1X_2	7.54	22.44	0.11	0.7419
X_1X_3	−172.13	22.44	54.84	<0.0001
X_1X_4	−142.61	22.44	40.39	<0.0001
X_2X_3	−30.54	22.44	1.85	0.1959
X_2X_4	−75.7	22.44	11.38	0.0045
X_3X_4	91.18	22.44	16.51	0.0012

anthocyanins in high temperatures could have been deteriorated and polymerized to form brown or colorless pigments. Due to stability of polymeric anthocyanins in different pHs, they cannot be measured by pH differential method, and as a result, total measured anthocyanins decreased (Giusti and Wrolstad 2001). At each temperature, loss of total anthocyanin content occurred with increasing extraction time to about 18 to 20 h, which could be due to the dominance of the damaging effects of temperature on the extraction of monomeric anthocyanins in the interval, and then, in 24-h extraction time, total anthocyanin content was stable or even slightly increased.

The interactive effect of solvent ratio and temperature on total anthocyanin content is shown in Fig. 2b. The maximum pigment content was extracted in 25 °C and in 80-ml/g solvent ratio. The higher solvent ratio made higher-density gradient and higher distribution coefficient, thereby resulting faster release from petal tissue and hence higher extracted anthocyanins. This trend continued till the solvent ratio reached to approximately more than 50 ml/g. With rising solvent proportion, due to lower solute weight ratio and decreasing the density of extracted anthocyanins, total anthocyanin content declined. Temperature around 45 °C deteriorated extracted anthocyanins; therefore, increasing the temperature irrespective of the solvent ratios reduced total anthocyanin content of the extract. Moreover, as previously mentioned, higher density of ingredients in extract at lower solvent ratios results in further reactions between monomeric anthocyanins and other compounds, thus declines the stability and shortens half-life of the anthocyanins.

Interaction between the temperature and ethanol concentration on the extraction rate (although it was not significant with 99 % confidence) is presented in Fig. 2c. As the surface plot shows, in general, increasing the percentage of ethanol and temperature reduced total anthocyanin content. Its reason could be the detrimental effects of temperature and ethanol (in the interval of 16 h) on total anthocyanin content of the samples. In another similar study conducted by Cacace and Mazza (2003b) on the anthocyanins extracted from blackcurrant, the interaction between ethanol percentage and temperature (150-min extraction time) was investigated on the anthocyanin content. They observed that by increasing the ethanol concentration (from 20 to 85 %), the critical temperature of anthocyanins (which is the temperature at which anthocyanins start to degrade) increased from 25 to 35 °C, and they reported that at all temperatures, with the rise in ethanol percentage up to 60 %, the extraction efficiency initially increased and then decreased. In another work of these two scientists (Cacace and mazza 2003a) carried out on the extraction optimization of phenolic compounds from milled berries, the highest amount of phenolic regardless of temperature was extracted in 60 % ethanol (150 min), and at higher or lower ethanol concentrations, the extraction rate decreased.

Differences between the results obtained in our study and others could be due to long time of extraction (16 h) as well as

Table 4 Predicted optimum conditions for the extraction of saffron petal anthocyanins and the response values

Factors	Low	High	Optimum
Solvent ratio (ml/g)	20	50	20
Ethanol concentration (%)	25	75	25.02
Extraction temperature (°C)	25	45	25.8
Time (h)	8	24	24
Total monomeric anthocyanin (mg/l)	1200	1680	1609.11

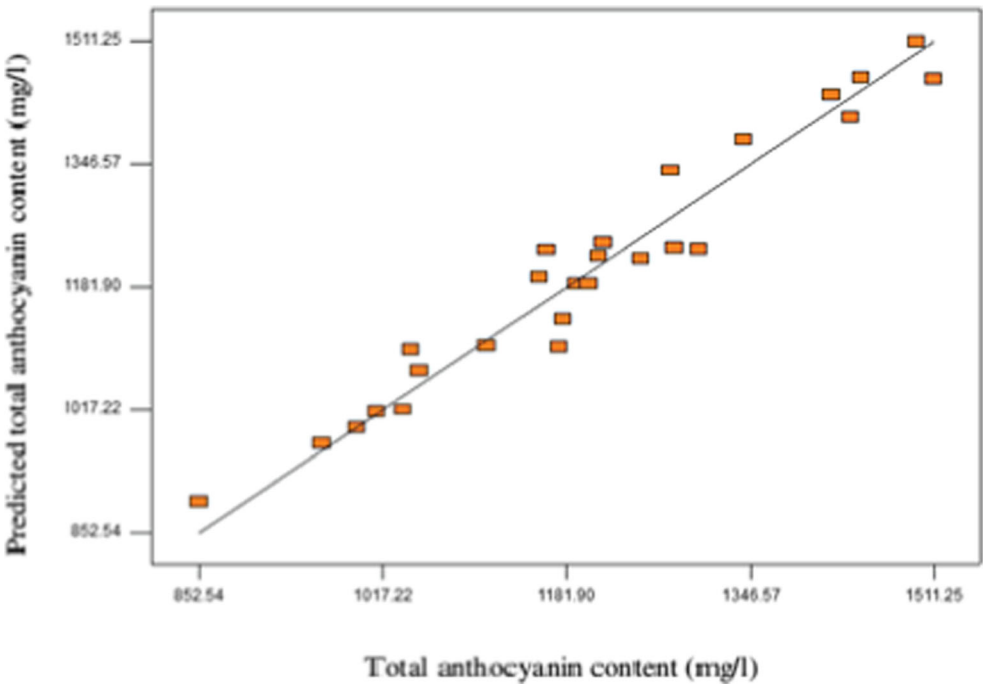

Fig. 1 Predicted and actual values of total anthocyanin content of saffron petal's extract

differences in the physical characteristics or chemical structure of the saffron petals.

In scrutinizing the effects of extraction time and solvent ratio (Fig. 2d), it was observed that the highest extraction efficiency was obtained in minimum time with maximum solvent ratio. In general, the most total anthocyanin content changes through different solvent ratios were observed between 8 and 12 h of extraction. Cacace and Mazza (2003b) reported that increasing solvent to solute ratio increased the amount of extracted phenolic compounds from blackcurrant and the time of extraction decreased. These researchers also found similar results in extraction of phenols from milled berries (Cacace and Mazza 2003a).

In Fig. 2e, the interaction between solvent ratio and ethanol concentration on total anthocyanin content (although it was not significant with 99 % confidence) is illustrated. As the chart indicates, lower ratios of solvent and higher percentages of ethanol led to a decrease in total anthocyanin content. Higher percentages of ethanol made a solution with lower polarity and thereby reduced the solubility of anthocyanins. As it can be seen in the surface plot, maximum total anthocyanin content was obtained approximately in 50 % ethanol and 50 ml/g of solvent to solute ratio. Cacace and Mazza (2003a) reported that increasing the ethanol concentration up to about 50 regardless of the solvent ratio, in extract of milled berries whereas transcending ethanol percent, reduced the extraction efficiency which have been followed in our study too.

Effects of the extraction time and ethanol concentration on total anthocyanin content of the extract have been displayed in Fig. 2f. According to the plot, total anthocyanin content was a function of both investigated variables. In all time intervals up to around 12 h, with increasing the ethanol percentage, the extraction rate increased, and in an opposite trend, in intervals more than 12 h of extraction, with increasing the ethanol percentage, total anthocyanin content was reduced. Therefore, the extract which was extracted in 24 h showed the highest total anthocyanin content by 25 % ethanol. In this diagram, totally at ethanol percentages more than 50, the declining trend of total anthocyanin content over time intervals had a higher speed in comparison with the lower concentrations of ethanol.

Optimization of the Anthocyanin Extraction

In order to achieve the highest level of anthocyanins (total anthocyanin content), optimal conditions of the extraction process were evaluated (Table 4). To define the variables, usage of the least amount of solvent and the least percentage of ethanol were considered. The lower limit of anthocyanin was equal to 1200 mg/l, which was less than approximately 60 % of the obtained data from different experiments. The upper limit of total anthocyanin content was considered as 1680 mg/l, which was equal to the total anthocyanin content obtained from two-time extraction (in 16 h, 35 °C, 50 % ethanol, and 50 ml/g solvent to petals) from the petals. Solution was provided by the software considering highest desirability, and the optimal extraction conditions were as follows: extraction time of 24 h, temperature of 25.8 °C, solvent ratio of 20 ml/g, and ethanol concentration of 25.02 % (Table 4). Under these conditions, the amount of extracted anthocyanin was 1609.11 mg/l.

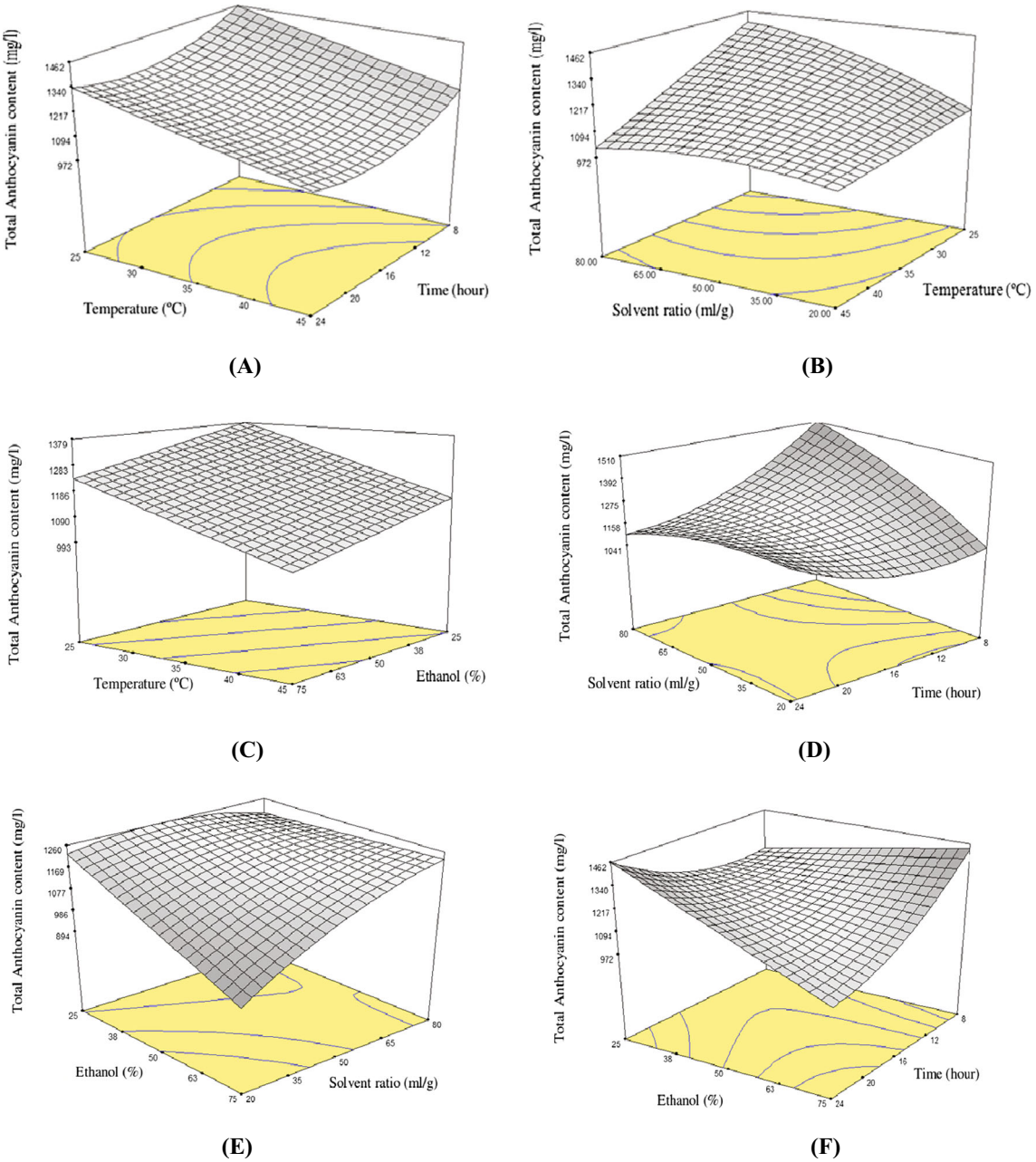

Fig. 2 Response surface plots for the effect of extraction parameters on total anthocyanin content of saffron petal's extract. **a** Temperature and time (ethanol 50 % and solvent ratio 50 ml/g), **b** temperature and solvent ratio (ethanol 50 % and temperature 35 °C), **c** temperature and ethanol percent (solvent ratio 50 ml/g and temperature 35 °C), **d** time and solvent ratio (ethanol 50 % and temperature 35 °C), **e** ethanol percent and solvent ratio (ethanol 50 % and solvent ratio 50 ml/g), and **f** ethanol percent and extraction time (temperature 35 °C and solvent ratio 50 ml/g)

In order to assess the compliance of the optimal extraction conditions with laboratory conditions, solvent extraction took place in the optimal extraction conditions provided by software. The average obtained anthocyanins from three replicates were equal to 1542.14 ± 67 mg/l (90.06 % of total anthocyanins in saffron petal). Considering standard deviation, obtained total anthocyanin content in lab conditions was close to the value predicted by the model and showed good fitness to the predicted data.

Conclusion

The results showed that the process variables, including temperature, extraction time, solvent ratio, and the percentage of ethanol, had statistically significant effects on anthocyanin extraction from the saffron petals. Quadratic polynomial model was fitted to the experimental data to predict the amount of extracted anthocyanins. Increasing temperature, regardless of the other variables, led to a reduction in the total anthocyanin

content. Rising the solvent ratio to the solute increased concentration gradient of anthocyanins and further increased the extraction efficiency. On the other hand, due to the low pH of the extract in high ratios of solvent to solute, the stability of extracted anthocyanins was increased. Total anthocyanin content was a function of both variables of ethanol percent and extraction time. In the first 12 h of extraction, as the percentage of ethanol solvent was increased, total anthocyanin content increased too. In contrast, in the second 12 h of extraction, total anthocyanin content decreased with increasing the ethanol concentration. Maximum monomeric anthocyanin content was obtained in 24 h extraction time, 25.8 °C, 20 ml/g solvent, and 25.02 % ethanol, and extracted anthocyanins was 1609.11 mg/l. Compared with other sources of natural anthocyanins, the saffron petal is very rich in anthocyanins and it can be used as a potential replacement for the natural color resources of anthocyanins for food and pharmaceutical industries.

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Compliance with Ethical Standards

Conflict of Interest Katayun Mahdavi Khazaei declares that he has no conflict of interest.

Seid Mahdi Jafari declares that he has no conflict of interest.

Mohammad Ghorbani declares that he has no conflict of interest.

Abbas Hemmati Kakhki declares that he has no conflict of interest.

Messiah Sarfarazi declares that he has no conflict of interest.

Human and Animal Rights We must include the following sentence to make sure that readers are aware that there are no ethical issues with human or animal subjects:

This article does not contain any studies with human or animal subjects.

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