Response surface optimization of conditions for debittering of white mahlab (*Prunus mahaleb* L.) juice using polystyrene resins

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Received: 4 February 2017 / Revised: 5 May 2017 / Accepted: 13 August 2017 / Published online: 12 December 2017
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Abstract In the current study, debittering of *white mahlab* (*Prunus mahaleb* L.) juice using polystyrene resins was investigated and optimized using the Box–Behnken response surface methodology. The effects of independent variables including resin type (cation exchange resin, anion exchange resin, and 50:50 cation/anion exchange resin), resin dosage (0.05, 0.075, and 0.1 g/mL) and agitation speed (50, 150, and 250 rpm) on total phenolic content, total flavonoid content, total anthocyanin content (TAC), antioxidant activity, and bitterness of *white mahlab* juice were studied. Effects of resin type and dosage were found to be significant ($p < 0.05$) for all responses. A dosage of 0.05 g/mL of the anion exchange resin and an agitation speed of 250 rpm were selected as the optimum conditions based on minimum bitterness (2.42) and maximum TAC (0.018 g/mol). We could conclude that the anionic resin can greatly reduce the intensity of bitterness and maintain the composition and characteristics of the *P. mahaleb* juice.

Keywords White mahlab juice · Debittering · Polystyrene resin · Response surface methodology

Introduction

Juices of berries with dark blue or red colors such as those of blackberries, strawberries, and blueberries are good sources of many biologically active compounds such as phenolic compounds, whose beneficial activities are primarily

ascribed to their antioxidant capacities [1, 2]. *White mahlab* (*Prunus mahaleb* L.) is a member of the *Rosaceae* family, which is also known as English cherry, Rock cherry, and St. Lucie cherry. The mahaleb cherry prefers a warm, dry climate and grows abundantly in West Asia; however, it is sometimes found in Eastern and Central Europe [3].

The debittering process is an effective solution to overcome commercial problems related to bitter taste of some fruits like grapefruit. Several debittering techniques such as enzyme treatment [4, 5], addition of bitterness suppressing agents [6, 7], ultrafiltration [8], aqueous treatment [9], and several types of resins [10–12] have been used to reduce or remove bitter compounds from various fruit juices. In the citrus processing industry, adsorbent resins have been successfully applied to remove bitter compounds because of their high selectivity for absorption. Several factors such as resin dosage, resin ratio, and agitation speed may affect the efficiency of the debittering process. To our knowledge, till date, no research has been conducted on the antioxidant capacities of *P. mahaleb* juice debittered using exchange resins.

Therefore, the purpose of this study was [1] to investigate the effect of resin type, resin dosage, and agitation speed on the natural bitterness and antioxidant activity (AA) of *P. mahaleb* juice and [2] to obtain optimal conditions to yield minimum bitterness and maximum total anthocyanin content (TAC), as main bioactive compounds in this fruit, using response surface methodology (RSM).

Materials and methods

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Steinheim, Germany). Folin–

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Ciocalteu reagent, gallic acid, sodium carbonate, aluminum chloride, methanol, and hydrogen peroxide were obtained from Merck (Darmstadt, Germany). The resins were purchased from Thermax Inc. (Pune, India).

Preparation of *P. mahaleb* juice

Ripe *P. mahaleb* L. fruits were collected from an orchard in the University of Zanjan, Zanjan province, Iran. The stems and leaves were discarded. The fruits were packed and sealed in polyethylene bags, transported to the laboratory, and then stored at $-18\text{ }^{\circ}\text{C}$ until use. The frozen fruits were removed from the freezer, thawed, and pitted. The fruit juice was obtained using a domestic juicer (Moulinex PC302, France). The juice was filtered through a stainless steel sieve (1 mm) to separate the pomace from the juice. The juice obtained was placed in dark glass bottles until further analyses.

Preparation of adsorbent resins

The ion exchange resins used in this study were TULSION A-2XMP ADM (macro-porous weak-base anion exchange resin) and TULSION T-42H (strong-acid cation exchange resin). The characteristics of the A-2XMP and T-42H resins were as follows: the functional groups were tertiary amines and nuclear sulphonic acids, respectively, and the particle size distribution of both was 0.3–1.2 mm. Each resin (cation exchange resin, anion exchange resin, and 50:50 cation/anion exchange resin) were pretreated by a series of steps according to the method described by Xu et al. [13] with minor modifications. Firstly, the resins were immersed in ethanol (95%) for 24 h, and then, the ethanol was washed completely using distilled water. Then, the resins were regenerated in sequence by immersing them in 5% HCl and 5% NaOH for 3 h. After each step of immersing, the resins were washed using distilled water to attain neutral pH.

The debittering process using resins

To optimize the dosage of the adsorbent resins, mixtures of a certain amount of each type of resin (0.05, 0.075, and 0.1 g) and 1 mL *P. mahaleb* juice sample were prepared and equilibrated in a thermostatic shaker at $25\text{ }^{\circ}\text{C}$ for 24 h under an agitation speed of 50, 150, and 250 rpm. Then, the juice was separated from the resins by filtering and collected for analyses [14, 15].

Determination of total phenolic content (TPC)

The TPC of the fruit juice was determined by the Folin–Ciocalteu colorimetric method [16]. Briefly, 40 μL of fruit

juice was mixed with 1.8 mL of the reagent (diluted 10 times with distilled water). After standing for 5 min at room temperature, 1.2 mL of sodium carbonate solution (7.5% w/v) was added. The solution was mixed and allowed to stand for 1 h at room temperature. Then, the absorbance was measured at 765 nm using a UV–visible spectrophotometer (SPECORD 250 UV–Vis, Germany). A calibration curve was prepared using a standard solution of gallic acid (Coefficient of determination or $R^2 = 0.99$). The results were expressed as mg of gallic acid equivalents per mL of juice.

Determination of total flavonoid content (TFC)

The TFC of the fruit juice was determined according to the colorimetric assay described by D’Abrosca et al. [17]. One mL of fruit juice (diluted 20 times using distilled water) was mixed with 0.3 mL of NaNO_2 (5% w/v). After 5 min, 0.3 mL of aluminum chloride (10% w/v) was added. After 6 min, 2 mL of NaOH (1 M) was added. Then, the volume was made up to 10 mL by the addition 6.4 mL of distilled water. The mixture was vigorously shaken, and the absorbance of the mixture was read at 510 nm. A calibration curve was prepared using a standard solution of rutin ($R^2 = 0.98$). The results were expressed as μg rutin equivalents per mL of juice.

Determination of TAC

The TAC was spectrophotometrically determined [18]. The *P. mahaleb* juice sample was mixed with acidified methanol (1:99 v/v), and the absorption was measured at a wavelength of 526 nm using the UV–visible spectrophotometer (SPECORD 250 UV–vis, Germany). TAC was calculated using Eq. (1):

$$A = \varepsilon \times B \times C, \quad (1)$$

where A is the absorbance of sample; B is the length of cell; ε is molar absorptivity ($\varepsilon = 33,000\text{ M}^{-1}/\text{cm}$); and C is the anthocyanin content expressed as mol/L.

Determination of DPPH radical scavenging activity

The free DPPH radical scavenging activity was spectrophotometrically evaluated according to the method described by Sharma et al. [19]. This assay is based on the principle that the purple color of DPPH changes to yellow on accepting a hydrogen atom (H) from the scavenger molecule, i.e., the antioxidant. Fruit juice (100 μL , diluted 20-fold using distilled water) was mixed with an aliquot of 3.9 mL of freshly prepared $6 \times 10^{-5}\text{ M}$ DPPH radical in methanol. The reaction tubes were wrapped in aluminum foil and kept in the dark at $25\text{ }^{\circ}\text{C}$ for 30 min. Absorbance

at 520 nm was measured using methanol as a blank. The inhibition of the DPPH radical was calculated according to the Eq. (2):

$$\text{Inhibition of DPPH radical (\%)} = [(A_0 - A)/A_0] \times 100, \quad (2)$$

where A_0 is the absorbance of DPPH (control sample) and A is the absorbance of the sample with DPPH.

Determination of H_2O_2 scavenging activity

The H_2O_2 scavenging activity of the fruit juice was determined according to the method of Boulekbache-Makhlouf et al. [20] with slight modifications. Fruit juice (1.5 mL) was mixed with 0.02 mL of 30% H_2O_2 . Absorbance was read at 530 nm. A decrease in the absorbance of the reaction mixture indicated an increase in scavenging activity. The percentage of inhibition of H_2O_2 was calculated using Eq. (3):

$$\text{Inhibition of } H_2O_2 = [(A_c - A_s)/A_c] \times 100, \quad (3)$$

where A_c is the absorbance of the control (without H_2O_2) and A_s is the absorbance in the presence of the sample.

Sensory evaluation

Sensory evaluation was performed by a sensory panel comprising eleven trained assessors (6 females and 5 males, aged 25–35 years). Firstly, the assessors fully rinsed their mouths with distilled water to allow an unbiased evaluation of the sensory attributes. Samples of *P. mahaleb* juice processed using a cation exchange resin, an anion exchange resin, and a 50:50 cation/anion exchange resins were compared with unprocessed *P. mahaleb* juice in terms of bitterness on a 5-point hedonic scale. The bitterness of the sample was scored as follows: 5 (very bitter), 4 (bitter), 3 (slightly bitter), 2 (bitter aftertaste), and 1 (without bitterness). The average of assessor's response was calculated as the score of each sample taste [21–23].

Experimental design and statistical analysis

In this study, the Box–Behnken RSM with three independent variables and three levels was used to design the experiment. Three main factors including resin type (A), resin dosage (B), and agitation speed (C) were chosen as the independent variables. The coded and uncoded levels of variables in the experimental design are shown in Table 1. The experimental conditions and the corresponding values are presented in Table 2.

The experimental data were fitted to the second-order polynomial model to obtain the regression coefficients (β).

Table 1 Coded and uncoded levels for the independent variables

Variable	Coded level		
	−1	0	1
A: Resin type	0	50:50	100
B: Resin dosage (g/mL)	0.05	0.07	0.10
C: Agitation speed (rpm)	50	150	250

The following generalized second-order polynomial model was used in the response surface analysis:

$$y = \beta_0 + \sum_{i=1}^K \beta_i X_i + \sum_{i=1}^K \beta_{ii} X_i^2 + \sum_{i \neq j=1}^K \beta_{ij} X_i X_j$$

where, y is the response variable, X_i and X_j are the independent variables, and k is the number of tested variables ($k = 3$). The regression coefficient is defined as β_0 for intercept, β_i for linear, β_{ii} for quadratic, and β_{ij} for cross terms. The analysis of variance was performed to determine individual linear, quadratic, and interaction regression coefficients. The fitness of the polynomial to the responses was estimated using the coefficient of determination (R^2). The significance of all the terms of the polynomial was statistically analyzed by computing the p value at $p < 0.05$, the coefficient of variance (CV%), and the predicted error of sum of square (PRESS). The experimental design, data analysis, and quadratic model building were conducted using the Stat-Ease Design-Expert 7.0.0 statistical software (Stat-Ease Inc., Minneapolis, MN, USA).

Validation of the model

The optimized conditions were validated for the maximum TAC and minimum intensity of bitterness based on the values obtained using RSM. The TAC and intensity of bitterness were determined under optimized conditions. The experimental values were compared with the predicted values to determine the validity of the model. The optimized condition was evaluated using additional triplicate experiments under optimal debittering conditions. The average value of the validation experiment was compared with the predicted value of the developed condition to determine the accuracy and suitability of the optimized conditions [24].

Results and discussion

Effect of debittering variables on TPC

The results of analyses revealed a significant ($p < 0.05$) linear (A and B), interactive (AB), and quadratic (A^2 and

Table 2 Experimental conditions with actual variable levels and the corresponding values

Run	Actual variable					Responses			
	Resin type	Resin dosage (g/mL)	Agitation speed (rpm)	TPC (mg/mL)	TFC (Hg/mL)	TAC (mmol/mL)	Bitterness	DPPH (%)	H ₂ O ₂ (%)
1	0	0.1	150	2.42	119.22	0.0089	2.43	27.16	4.06
2	0	0.075	50	2.42	119.53	0.01	2.46	27.42	4.83
3	50	0.1	250	2.67	143.8	0.0059	2.85	28.48	5.64
4	100	0.075	50	2.88	161.11	0.0045	3.51	30.52	6.84
5	50	0.05	250	2.73	157.56	0.0079	3.12	29.83	6.65
6	50	0.075	150	2.68	149.36	0.0068	2.98	28.53	5.76
7	0	0.075	250	2.42	119.94	0.012	2.44	27.43	4.86
8	50	0.075	150	2.67	148.76	0.0064	2.97	28.58	5.76
9	0	0.05	150	2.50	139.21	0.017	2.63	28.16	5.42
10	0	0.075	250	2.88	161.82	0.0047	3.52	30.53	6.85
11	100	0.1	150	2.88	160.1	0.0040	3.51	29.91	6.73
12	50	0.05	50	2.71	157.32	0.0075	3.11	29.83	6.64
13	50	0.1	50	2.67	144.3	0.0055	2.89	28.47	5.60
14	50	0.075	150	2.67	148.52	0.0061	2.96	28.58	5.77
15	100	0.05	150	2.90	164.8	0.005	3.91	31.97	7.19

B²) effect of the debittering process variables on the TPC. Based on the regression coefficient (β) values, the type of resin (A) showed a major positive effect. The non-significant variables were removed from the model, and the final reduced second-order polynomial is shown in Table 3.

The non-significant value of the lack of fit ($p > 0.05$) test, the value of PRESS (0.004), a high value of coefficient of determination ($R^2 = 0.99$), and a low coefficient of variation ($CV = 0.37\%$) indicated that the model is well fitted.

A three-dimensional response surface plot was generated to visualize the effect of the debittering process variables on the TPC. The response surface plot for TPC as function of two independent variables, resin dosage, and resin type with agitation speed as the third independent variable was fixed at the central experimental level of zero [Fig. 1(A)]. The results showed that increasing resin dosage decreased the TPC, which is in agreement with the results obtained by Ibeas et al. [25] and Bao et al. [15]. At a fixed resin dosage and agitation speed and among the three

different types of resins tested, the TPC decreased more with the A-2XMP resin. This might be due to the low adsorption ratio and low affinity of the analyzed compounds with the cation exchange resin [26, 27]. Resin performance is affected by chemical structure and surface functionality. The binding of phenolic compounds to ion exchange resins is affected by interactions between functional groups, hydrophobic interactions, and hydrogen bonding. These interactions with anion exchanger resins showed different mechanisms at different pH values. Adsorption and ion exchange were predominant under alkaline conditions, whereas phenol desorption was predominant at acidic pH. At acidic pH, the uptake of phenolic compounds by different adsorbents is enhanced because the phenols are undissociated and the dispersion interactions predominate [28].

In addition, the effect of resin treatment on the TPC was in agreement with literature data for grapefruit juices [29]. It was pointed out that the debittering process using Amberlite-IRA-400 ion exchange resin led to a decrease in

Table 3 The regression models for TPC, TFC, TAC, bitterness, DPPH, and H₂O₂ using independent variables: resin type (A), resin dosage (B), and agitation speed (C)

Response	Model	R ²
TPC	$+2.68 + 0.22A - 0.027B + 0.015AB - 0.021A^2 + 0.024B^2$	0.99
TFC	$+0.15 + 0.019A - 0.006B + 0.003AB - 0.0064A^2 + 0.0036B^2$	0.98
TAC	$+0.0065 - 0.0037A - 0.0016B + 0.0017AB + 0.0016A^2$	0.94
Bitterness	$+2.97 + 0.56A - 0.14B - 0.051AB + 0.068A^2 + 0.078B^2 - 0.057C^2$	0.99
DPPH	$+28.57 + 1.59A - 0.72B - 0.26AB + 0.28A^2 + 0.46B^2 + 0.13C^2$	0.99
H ₂ O ₂	$+5.77 + 1.05A - 0.48B + 0.23AB - 0.1A^2 + 0.19B^2 + 0.18C^2$	0.99

Fig. 1 Response surface plots (3D) illustrating the effect of resin type and resin dosage (g/ml) on (A) TPC, (B) TFC, (C) TAC, (D) bitterness, (E) DPPH, and (F) H₂O₂

the TPC (21%) compared with samples of fresh grapefruit juices [29].

Effect of debittering variables on TFC

The linear (A and B), interactive (AB), and quadratic (A^2 and B^2) effect of the debittering process variables had significant ($p < 0.05$) effect on the TFC of *P. mahaleb* juice.

Based on regression coefficient (β) values, the type of resin (A) showed a major positive effect. The non-significant variables were removed from the model, and the final reduced second-order polynomial is shown in Table 3.

A high value of coefficient of determination ($R^2 = 0.98$), a PRESS value of 0.0002, and a low value of coefficient of variation ($CV = 1.58\%$) for the predicted model were observed.

A response surface plot for TFC as a function of resin dosage and resin type is given in Fig. 1(B). TFC was greatly decreased as the resin dosage increased from 0.05 to 0.1 (g/mL). Among the three different types of resins tested, the TFC decreased to a higher degree with the A-2XMP resin when the resin dosage and agitation speed were kept constant at the middle ranges (0.07 g/mL and 150 rpm, respectively). This result is in agreement with those reported by Bretage et al. [30] and Kammerer et al. [31]. They claimed that the anionic resin is highly effective for absorbing flavonoid compounds.

Effect of debittering variables on TAC

The TAC of the *P. mahaleb* juice is shown in Table 2. The results of analyses showed a significant ($p < 0.05$) resin dosage (B), resin type (A), interactive (AB) and quadratic (A^2) effect of the debittering process variables on the TAC. The non-significant variables were removed from the model, and the final reduced second-order polynomial is shown in Table 3.

A high value of the coefficient of determination ($R^2 = 0.94$), a PRESS value of 0.00003, and a coefficient of variation (CV) of 13.11% for the predicted model were observed. The lack of fit was not significant ($p > 0.05$), indicating that the model could adequately fit the experimental data.

The response surface plot showing the influence of the resin dosage and resin type on the TAC is shown in Fig. 1(C). The TAC decreased with an increase in the amount of cation exchange resin (T-42H), which is in conformity with the results obtained by other researchers [32].

Lasanta et al. [33] and Ibeas et al. [25] reported that the content of individual anthocyanins significantly decreased with an increase in the percentage of wine treated with the

cation exchange resin. A high loss of these pigments is probably due to pigment retention on the resin surface.

Effect of debittering variables on bitterness score

The mean scores of the sensory attributes for the *P. mahaleb* juice are shown in Table 2. The final reduced model for the bitterness score was rendered by considering only the significant coded terms as shown in Table 3.

A high value of coefficient of determination ($R^2 = 0.99$) and a PRESS value of 0.079 with a low value of coefficient of variation ($CV = 1.09\%$) and a non-significant value of lack of fit ($p = 0.07$) showed that the model is well fitted.

The removal of bitterness as a function of resin dosage and resin type is presented in Fig. 1(D). A high removal of bitterness was achieved by usage of 0.075 g A-2XMP. Furthermore, increasing the dosage of resin beyond 0.075 g gave only a slight increase in the removal of bitterness.

Ion exchange resins were found to be promising alternatives for successful removal of the bitter compounds from citrus juices [12, 34–36]. In the present study, the bitterness decreased more with the anion exchange resin, A-2XMP, which was in agreement with the results of Johnson and Chandler [37] that applied two anion exchange resins, IRA-401s and A 378, to combine the debittering and deacidifying function processes for citrus juices.

Effect of debittering process variables on AA

The result showed significant ($p < 0.05$) linear (A and B), interactive (AB), and quadratic (A^2 , C^2 , and B^2) effect of the process variables on antioxidant activity of debittered *P. mahaleb* juice.

The final reduced model for AA of the *P. mahaleb* juice was generated by considering only the significant coded terms as shown in Table 3.

The results of the multiple regression analyses of the models were significant ($p < 0.05$). The lack of fit of the models of DPPH was not significant ($p > 0.05$), but it was significant ($p < 0.05$) for H_2O_2 . Although the lack of fit was found to be significant for H_2O_2 , a low coefficient of variance was observed for H_2O_2 ($CV = 1.08\%$). The coefficients of determination for DPPH and H_2O_2 were 0.9986 and 0.9971, respectively. The value of PRESS for DPPH and H_2O_2 were 0.32 and 0.29, respectively.

The variation of the AA with resin dosage and resin type is represented in Fig. 1(E, F). It is observed that the AA decreased with increasing dosage of the resin, in particular, the A-2XMP resin.

The AA of fruit juices is largely due to the presence of phenolic compounds. In general, the phenolic compounds are able to scavenge radicals and to chelate metals.

However, it depends on the concentration and the type of the phenolic compounds [38]. In this sense, quantitative and qualitative variations of these compounds (e.g., alteration of one or more hydroxyls or oxidation) may have occurred during the debittering treatment leading to differences in the scavenging activity of the juices [39].

Determination and experimental validation of optimal conditions

The results of the numerical optimization showed the following optimal conditions: resin type: A-2XMP; resin dosage: 0.05 g/mL; and agitation speed: 250 rpm. Under these optimal conditions, the experimental values were in agreement with the predicted values. The experimental values for TAC and the bitterness score were 0.018 and 2.42, respectively. The predicted values for TAC and the bitterness score were 0.015 and 2.58, respectively.

From the current study, we concluded that resin dosage and resin type were the most important factors affecting optimization of debittering conditions of *P. mahaleb* juice, whereas agitation speed did not have a significant effect on the optimization of debittering conditions ($p < 0.05$). The TPC, TFC, and AA decreased with increasing of resin dosage (g/mL) of the anion exchange resin A-2XMP. The optimum conditions to obtain minimum bitterness and maximum TAC were as follows: resin type: A-2XMP; resin dosage: 0.05 g/mL; and agitation speed: 250 rpm. Under these conditions, the bitterness score of 2.58 and a TAC of 0.015 (mmol/L/g) were obtained. It could be concluded that the application of an anion exchange resin is an interesting method to improve the bitterness of *P. mahaleb* juice, as well as to improve the general quality of the product.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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