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Influence of Ultrasound-Assisted Extraction on Bioavailibity of Bene Hull (*Pistacia Atlantica* Subsp. *Mutica*) Extract: Testing Optimal Conditions and Antioxidant Activity

Mojtaba Delfanian[1], Mohammad Hossein Haddad Khodaparast[2*], Mohammad Ali Razavi[2], Reza Esmaeilzadeh Kenari[3]

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Abstract

The central composite rotatable design by response surface methodology was applied for optimization of ultrasonic extraction conditions of Bene hull (*Pistacia atlantica* subsp. *Mutica*) polyphenols. The sonication time, temperature and ethanol-water ratio were independent parameters studied for the extraction optimization. Total polyphenols and antioxidant potentials of extracts in terms of ferric reducing antioxidant potential (FRAP), DPPH scavenging activity and oxidative stability index (OSI) were determined. The obtained data were well consistent with the polynomial equations by significant variation in linear, quadratic and interaction impacts of the process factors. The optimized extraction conditions were sonication time, 26.91 min, temperature, 50.42 °C and ethanol concentration, 55.84%. The total polyphenols, DPPH, FRAP an OSI of optimal extract were 304.47 mg GAE/g, 72.47%, 54.04 mmol/100g and 8.55 h, respectively. High performance liquid chromatography (HPLC) analysis of optimal extract detected presence of epicatechin, chlorogenic, sinapic, caffeic and gallic acids.

Keywords: Antioxidant activity; Bene hull; Polyphenols; Response surface methodology; Ultrasound-assisted extraction.

Introduction

Polyphenols such as flavonoids are important bioactive compounds in terms of antioxidant activity, antimicrobial activity and etc., in plants (Delfanian *et al.* 2016). The addition of antioxidants is effective to terminate or delay oxidation process by chelating free catalytic metals, scavenging free radicals and also by acting as electron donors (Anagnostopoulou *et al.* 2006). Many countries such as Canada and America have prohibited use of synthetic antioxidants (BHA, BHT and TBHQ) in food lipids due to increasing of cancer risk, so plants natural antioxidants can be used as a suitable alternative (Delfanian *et al.* 2015).

Pistacia atlantica belonging to the family of Anacardiaceae and has various subspecies: *mutica*, *kurdica*, *atlantica* and *cabulica*. Bene (*Pistacia atlantica* subsp. *Mutica*) tree grows in dry and semi dry regions of Iran such as Kerman, Khorasan and Sistan-Baluchestan provinces (Farhoosh *et al*. 2009). Bene is useful for treatment of the liver, spleen, night-blindness, peptic ulcer and rickets (Shaddel *et al.* 2014). Several studies confirmed the biological activity of Bene hull bioactive compounds such as anti-inflammatory, antimicrobial, antitoxic and antioxidant activities (Gourine et al. 2010, Hatamnia *et al*. 2014). Recent researches on Bene mainly considered the fatty acids, phytosterols, triacylglycerol and essential oils composition (Benhassaini *et al*. 2007, Farhoosh *et al*. 2008).

Ultrasound-assisted extraction (UAE) comparing to other extraction methods such as supercritical fluids, superheated water, accelerated solvent and microwave

1 And 2. PhD Student and Professor, Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Associated professor, Department of Food Science and Technology, Faculty of Agricultural Engineering, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
(*-Corresponding Author: khodaparast@um.ac.ir)
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has many benefits including simplicity, shorter time and high efficiency (Xie *et al*. 2012). The cavitation generated in the solvent during sonication and thermal impacts lead to destruction of cell wall and increase the extraction efficiency (Xu and Pan *et al*. 2013). Different extraction parameters including solvent polarity, time, temperature, liquid-to-solid ratio and etc., are effective in extraction process of bioactive compounds (Liew *et al*. 2005).

Response surface methodology (RSM) is an effective statistical and mathematical tool for optimization of process conditions which can describe the effect of independent variables on response values. Recently, RSM is applied for optimization of antioxidants extraction conditions from various sources (Da Porto et al. 2013, Li *et al*. 2015, Rodríguez-Pérez *et al*. 2015, Szydłowska-Czerniak and Tułodziecka 2015). Currently, there is no available scientific document about optimization of UAE of phenolic compounds from Bene hull by RSM. Therefore, in the present study RSM was used for optimization of extraction parameters ethanol-water ratio, temperature and sonication time during ultrasonic irradiation in order to maximize antioxidant capacity and polyphenols content from Bene hull.

Materials and methods

Chemicals

All the solvents and chemicals used were of analytical or HPLC grade. Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate anhydrous (Na_2CO_3), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), iron (III) chloride anhydrous, 2,4,6-tripyridyl-s-triazine (TPTZ) and HPLC standards were purchased from Merck Co. (Darmstadt, Germany). Ethanol and hydrochloric acid (HCl) were obtained from Scharlau Co. (Barcelona, Spain).

Plant Materials

Bene fruits were collected in August 2015 from the fields of Khvaf, Razavi Khorasan, Iran. After air-drying (at 30°C for 72 h in shadow), the green hulls of samples were separated using a mechanical instrument. Samples were frozen in the dark at –18 °C for further experiments (Rezaie et al. 2015).

Ultrasound-Assisted Extraction (UAE)

The UAE was carried out in an ultrasonic bath (DT 102H, Bandelin, Germany) at 35 kHz (100% power). Dried samples (50 g) were placed into Erlenmeyer flasks and extracted with 250 mL of different ratios of aqueous ethanol (0-100%) at various temperatures (25-65°C) and times (varying from 5 to 50 min). The mixtures were filtered and evaporated at 35°C to remove solvents using a vacuum oven. Finally, concentrated samples were stored at -18°C (Hammi *et al.* 2015).

Determination of total polyphenols

Total polyphenols content (TPC) of samples were determined using Folin–Ciocalteu assay as described by *Sfahlan et al*. (2009). Briefly, 0.1 mL of different extracts (1 mg/mL) was mixed with 2.5 ml of 10-fold-diluted Folin-Ciocalteu reagent. The solution was mixed thoroughly and allowed to stand at room temperature. After 4 min, 2 mL of 7.5% sodium carbonate solution was added and then incubated at 45°C for 15 min. The estimation of phenolic compounds was done at 765 nm using a UV–Vis spectrophotometer (Model 160A Shimadzu, Japan) and calculated by a calibration curve (R^2=0.99) performed with gallic acid (0 to 0.4 mg/mL). The TPC was expressed as mg of gallic acid equivalents (GAE) per g of dried sample.

Determination of antioxidant capacity
DPPH Method

The ability of samples to scavenge DPPH$^{\bullet}$ radicals was evaluated following the procedures described by Delfanian *et al*. (2015). This parameter was assessed according to ability of the extracts to reduce free radicals. Accurately, 5 mL of DPPH$^{\bullet}$ ethanolic solution (0.004%) was mixed with 50 µL of extract (0.5 mg/mL) and the reaction mixture was shaken vigorously and incubated in the dark at ambient temperature for 30 min. The absorbance of the mixtures was estimated

at 517 nm against a blank. The radical scavenging activity of the extracts was expressed as a percentage of DPPH$^{\bullet}$ radical attraction calculated according to Eq. (1) below:

$$\%\ \text{Inhibition} - \left[1 - \frac{Abs_{sample}}{Abs_{blank}}\right] \times 100 \quad (1)$$

FRAP Method

The ferric reducing antioxidant power assay followed was according to Sulaiman et al. (2011). The FRAP reagent was prepared by mixing 300 mM sodium acetate anhydrous in distilled water pH 3.6, 20 mM ferric chloride hexahydrat in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-s-triozine (TPTZ) in 40mM HCl in a proportion of 10:1:1. Then, 50 µL of diluted sample extract (0.5 mg/mL) was mixed with 50 µL distilled water and 900 µL of FRAP reagent. The absorbance of the solution was measured at 593 nm against a blank after 30 min incubation at 37 °C. In the case of the blank, 100 µL of distilled water was added to 900 µL of FRAP reagent. Calibration curve was prepared using Iron (II) sulfate ($FeSO_4$) at concentrations from 30 to 1000 µmol/mL. The results were expressed as mM of Fe^{+2}/100 g extract. All tests were carried out in triplicate.

Oxidative Stability Index (OSI)

Rancimat (Metrohm 743, Herisau, Switzerland) was applied for measurement of OSI. The test was performed at 110°C and an airflow rate of 15 l/h (3g refined soybean oil, containing 1000 ppm of extract) (Rezaie *et al*. 2015).

HPLC Analysis

Samples were analyzed according to the method approved for identification of polyphenols in olive oil by International olive Council (COI/T.20/Doc No29. 2009). The HPLC system which was used in this study was a Younglin (South Korea) equipped with an UV/Vis detector (Younglin, South Korea). The phenolic compounds in a 10 µL of sample solution were separated on a Hector C-18 column (150×4.6 mm, 5 µm) at room temperature and detected at 280 nm. The mobile phase consisted of solvent A (water-phosphoric acid, 0.2%) and solvent B (methanol-acetonitrile, 50%). Solvent gradient was used in four steps: 25 min, 4-50% B; 5min, 50-60% B; 25 min, isocratic elution of 100% B; back to initial status for two minutes. The total elution time flow rate was 72 min and 1.0 mL/min, respectively.

Experimental Design

Using the Design-Expert Version 6.0.2 software (Stat-Ease, Inc., USA) response surface methodology was applied for optimization of UAE parameters based on central composite rotatable design (CCRD). The effects of process factors: sonication time (X_1; min), temperature (X_2; °C) and ethanol concentration (X_3; %) were investigated on four dependent variables (as responses), namely TP, DPPH, FRAP and OSI. Table 1 is shown the experimental designs of the coded and un-coded extraction factors.

Table 1- Coded and uncoded levels of independent variables employed for optimization of the extraction of polyphenols

Independent variables	Symbols	Coded levels		
		-1	0	+1
Time (min)	X_1	5	27.5	50
Temperature (°C)	X_2	25	45	65
Ethanol concentration (%)	X_3	0	50	100

Range of sonication time, temperature and ethanol-water ratio was chosen based on preliminary experiments. Data was achieved from CCRD fitted by a second-order polynomial equation as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=0}^{n} \sum_{j=2}^{3} \beta_{ij} X_i X_j \quad (2)$$

Where Y is the dependent factor, β_0, β_i, β_{ii} and β_{ij} are the coefficients for intercept, linear, quadratic and interaction, respectively and X_1, X_2, and X_3 represent the independent factors. The model fitness was estimated by analyzing of coefficient R^2, adjusted coefficient R^2_{Adj}, lack of fit and analysis of variance (ANOVA). All tests were done in triplicate and confidence level was 95.0%.

Results and discussion

Model fitting using RSM

The impacts of independents parameters including ethanol-water ratio, temperature and time under ultrasound-assisted extraction on responses were investigated by CCRD of RSM. Table 2 shows the experimental design and response values of TPC, FRAP, DPPH and OSI determined for Bene hull extracts. Experimental responses obtained from the CCRD were fitted into the second-order polynomial models and coefficients R^2 of the calculated equations were investigated by ANOVA. The adequacy of the model is determined by F-test, lack of fit, coefficients R^2, predicted R^2, adjusted R^2 and *P*-value (Yim et al. 2012). The ANOVA results indicated lower *P*-values with higher R^2, R^2_{adj} and R^2_{pre} (> 0.8) associated insignificant lack of fit (P>0.05) for experimental responses, show that there was an appropriate relationship between the response and independent factors (Tables 3 and 4). Regression coefficients R^2 for TPC, DPPH, FRAP and OSI were 0.9709, 0.9371, 0.9304 and 0.9473, respectively.

Response Surface Analysis

As it can be seen in Table 3, the response surface analysis (RSA) of the experimental results indicates that all three factors; sonication time, temperature and solvent ratio have quadratic effect on phenolic content with an appropriate coefficient R^2 (0.9709). The predicted data TPC for total phenolic content of extracts were calculated with the following equation:

$$TPC = 304.74 + 18.32X_3 - 114.32X_1^2 - 30.75X_2^2 - 45.4X_3^2 + 14.68\ X_1X_3 + 18.79X_2X_3 \quad (3)$$

Ethanol concentration (X_3) was only variable by significant linear impact ($P < 0.05$), while the variables sonication time (X_1), temperature (X_2) and ethanol concentration had quadratic impacts on TPC. Also, RSA revealed that interaction between variables time and solvent ratio and also temperature and solvent ratio were significant, whereas reciprocal interaction of time and temperature was not significant.

Table 2- Response surface central composite design, experimental and predicted responses for the dependent variables

Test	Independent variables			Dependent variables (Response)							
	Time (min), X_1	Temp (°C), X_2	Ethanol (%), X_3	Phenols (mg GAE/g)		DPPH (% Inhibition)		FRAP (mM of Fe^{+2}/100g)		OSI (h)	
				Expt.	Pred.	Expt.	Pred.	Expt.	Pred.	Expt.	Pred.
1	5.00	25.00	0.00	118.54±2.98	129.41	9.21±1.71	4.99	17.26±0.85	15.39	11.27±0.08	11.57
2	27.50	45.00	50.00	310.25±3.86	304.74	72.26±0.60	70.41	59.06±0.79	53.08	8.88±0.17	8.68
3	50.00	25.00	0.00	123.06±4.91	100.04	28.28±1.54	21.90	15.03±0.63	15.39	11.94±0.66	11.57
4	5.00	25.00	100.00	96.73±3.46	99.11	25.31±0.88	23.33	25.91±1.08	24.71	8.67±0.42	8.42
5	5.00	65.00	100.00	130.26±2.56	136.69	57.07±0.84	53.45	31.76±0.76	32.56	8.84±0.31	8.89
6	50.00	45.00	50.00	182.16±2.17	190.41	43.26±0.57	51.50	30.87±0.98	30.63	8.18±0.29	8.68
7	27.50	45.00	0.00	246.36±3.82	241.01	44.56±0.45	47.92	31.56±1.31	33.91	11.15±0.19	11.34
8	27.50	25.00	50.00	245.26±3.90	273.98	42.26±0.37	54.85	42.53±1.71	53.08	8.45±0.02	8.68
9	50.00	65.00	100.00	170.62±5.39	166.05	38.09±0.79	36.54	34.80±0.94	32.56	9.25±0.02	8.89
10	5.00	65.00	0.00	78.25±3.19	91.83	30.36±1.21	35.11	9.65±1.92	7.54	11.45±0.05	11.10
11	27.50	45.00	50.00	318.94±2.02	304.74	70.36±0.33	70.41	61.25±0.87	53.08	8.49±0.31	8.68
12	27.50	45.00	50.00	308.35±2.66	304.74	74.56±0.33	70.41	54.51±1.88	53.08	8.58±0.55	8.68
13	27.50	45.00	50.00	328.42±4.76	304.74	78.08±0.44	70.41	52.06±1.57	53.08	8.82±0.34	8.68
14	27.50	65.00	50.00	282.12±4.92	273.98	70.12±0.81	68.05	45.15±1.19	53.08	8.92±0.01	8.68
15	27.50	45.00	50.00	301.12±3.58	304.74	78.36±0.25	70.41	58.12±1.66	53.08	8.93±0.23	8.68
16	50.00	65.00	0.00	58.55±5.88	62.47	15.72±0.61	18.20	6.26±1.45	7.54	10.87±0.47	11.10
17	27.50	45.00	100.00	251.72±6.86	277.66	59.10±0.68	66.26	47.53±1.95	51.08	8.12±1.64	8.66
18	50.00	25.00	100.00	158.65±3.81	128.48	40.26±1.92	40.24	25.62±0.53	24.71	8.40±0.04	8.42
19	5.00	45.00	50.00	178.08±3.24	190.41	49.23±0.67	51.50	24.48±1.19	30.63	8.63±0.34	8.68
20	27.50	45.00	50.00	302.52±3.24	304.74	69.88±0.45	70.41	57.84±1.20	53.08	8.92±0.23	8.68

Table 3- Analysis of variance (ANOVA) of the quadratic model adjusted to the total phenolic content and DPPH• scavenging activity assays

Squares	Sum of Square	DF	Mean Square	F Value	P-value Prob F
Total phenolic content					
Model	1.489E+005	6	24817.46	72.29	< 0.0001
X_3	3356.96	1	3356.96	9.78	0.0080
X_1^2	35941.64	1	35941.64	104.69	< 0.0001
X_2^2	2600.76	1	2600.76	7.58	0.0165
X_3^2	5668.87	1	5668.87	16.51	0.0013
X_1X_3	1724.61	1	1724.61	5.02	0.0431
X_2X_3	2823.76	1	2823.76	8.23	0.0132
Residual	4462.93	13	343.30		
Lack of Fit	3921.48	8	490.19	4.53	0.0565
Pure Error	541.45	5	108.29		
Cor Total	1.534E+005	19			
R^2	0.9709				
Adj.R^2	0.9575				
Pred.R^2	0.9138				
DPPH• scavenging activity					
Model	8168.56	6	1361.43	32.30	< 0.0001
X_2	436.13	1	436.13	10.35	0.0067
X_3	840.89	1	840.89	19.95	0.0006
X_1^2	982.94	1	982.94	23.32	0.0003
X_2^2	220.82	1	220.82	5.24	0.0395
X_3^3	487.98	1	487.98	11.58	0.0047
X_1X_2	571.90	1	571.90	13.57	0.0028
Residual	547.87	13	42.14		
Lack of Fit	478.69	8	59.84	4.32	0.0618
Pure Error	69.18	5	13.84		
Cor Total	8716.42	19			
R^2	0.9371				
Adj.R^2	0.9081				
Pred.R^2	0.8172				

Fig.1A shows the reciprocal interaction effect of sonication time and ethanol-water ratio on the TPC. TPC increased by increasing ethanol concentration to 50%, while it increased with extraction time until 27.5 min and then declined, confirming reverse quadratic impact of solvent ratio and time. Moreover, this plot demonstrates the positive reciprocal interaction impacts of solvent ratio and time on TPC. As clearly seen in Fig. 1B, at 50% aqueous ethanol, the total polyphenols increased by increasing temperature to 45 ℃, and then decreased at higher temperatures (>45°C). In general, maximum of polyphenols (328.42 mg GAE/g) was extracted with 50% aqueous ethanol, at 45 ℃ for 27.5 min.

Water can conveniently penetrate into the plant cells, while protein is denatured in high proportion of ethanol and prevents the dissolution of polyphenols (Yang *et al*. 2010). Water is not an appropriate solvent for extraction of carbonaceous compounds, hence mixture of water and alcohols can increase the extraction efficiency (Delfanian *et al*. 2015). According to the ‘‘like dissolves like’’ principle, extraction efficiency of polyphenols increased by increasing of solvent polarity (Zhang *et al.* 2007, Zhang *et al*. 2008). We found that the recovery of polyphenols was higher in mixtures of ethanol/ water (1:1) compared to pure ethanol and water. These results were in agreement with the results reported by Hemwimol *et al*. (2006); Delfanian et al. (2015) and Hammi *et al*. (2015).

DPPH• scavenging activity is a valid and reliable assay for evaluation of antioxidant properties of extracts (Li *et al.* 2006). According to ANOVA results there was a quadratic relationship between DPPH and sonication variables with high coefficient R^2 (0.9371) (Table 3). The following Eq. (4) demonstrates the real model for the DPPH• scavenging ability:

$$DPPH = 70.41 + 6.6X_2 + 9.17X_3 - 18.91X_1^2 - 8.96X_2^2 - 13.32X_3^2 - 8.46X_1X_2 \quad (4)$$

DPPH equation indicates that the sonication temperature and ethanol concentration were linear effects and all three variables were quadratic effects on response. Also, there was a significant interaction among irradiation time and temperature (P<0.05). The model was fitted and adequate for DPPH with non-significant lack of fit and high coefficients R^2 (Table 3). According to Fig. 1C the DPPH inhibition declined with rising process time at shorter or longer durations than 27.5 min, supporting the reverse quadratic impact of time. Generally, our results revealed that the highest value of DPPH$^{\bullet}$ inhibition was obtained with 50% ethanol, at 45 °C for 27.5 min. In order to minimize process time and cost-saving may be preferred combination of the lowest levels of extraction parameters in the optimum zone. This result were in agreement by MorelliPrado (2012); *Yim et al*. (2012) and Setyaningsih et al. (2016) that noted the highest DPPH$^{\bullet}$ inhibition in extracts was obtained in moderate extraction time and temperature.

The real model correlating the FRAP in term of significant independent variables is given below:

$$FRAP= 53.08+ 8.59\ X_3 – 22.45X_1^2 – 10.58X_3^2 + 3.93X_2X_3 \quad (5)$$

FRAP equation shows that the irradiation time and ethanol concentration were quadratic impacts, whereas solvent variable had also a linear effect on FRAP values. There was a significant interaction among ethanol concentration and temperature at 95% confidence level.

According to ANOVA results (Table 4) model were significant and valid for FRAP values with non-significant lack of fit and high regression coefficient. Therefore, model can be applied for prediction of data as respects there was a high correlation between the predicted and experimental data.

Table 4- Analysis of variance (ANOVA) of the quadratic model adjusted to the FRAP and OSI assays

Squares	Sum of Square	DF	Mean Square	F Value	P-value Prob F
FRAP					
Model	5365.01	4	1341.25	50.10	< 0.0001
X_3	737.19	1	737.19	27.54	< 0.0001
X_1^2	1612.72	1	1612.72	60.24	< 0.0001
X_3^2	358.15	1	358.15	13.38	0.0023
X_2X_3	123.32	1	123.32	4.61	0.0486
Residual	401.55	15	26.77		
Lack of Fit	346.80	10	34.68	3.17	0.1076
Pure Error	54.75	5	10.95		
Cor Total	5766.56	19			
R^2	0.9304				
Adj.R^2	0.9118				
Pred.R^2	0.8987				
OSI					
Model	27.07	3	9.02	95.84	< 0.0001
X_3	17.96	1	17.96	190.71	< 0.0001
X_3^2	8.66	1	8.66	91.97	< 0.0001
X_2X_3	0.46	1	0.46	4.84	0.0428
Residual	1.51	16	0.094		
Lack of Fit	1.33	11	0.12	3.41	0.0932
Pure Error	0.18	5	0.035		
Cor Total	28.58	19			
R^2	0.9473				
Adj.R^2	0.9374				
Pred.R^2	0.9112				

Fig. 1D illustrates the level of FRAP was increased by increasing of ethanol-water ratio up to 50% and degrades at high ratio of ethanol during long extraction times. The highest FRAP value was observed under the center point variables (50% aqueous ethanol at 45°C for 27.5 min). These results were in agreement by

Moyo *et al.* (2003) and Yim *et al.* (2012) whom explained linear effects of extraction variables are less than their interactions which occurs in reality.

Rancimat assay is often applied for estimate the oxidative stability index (OSI) of samples based on changes in water electrical conductivity resulting from the production of volatile acids such as formic acid (Farhoosh *et al*. 2009). Longer oxidative stability index values demonstrate higher antioxidant ability. The obtained mathematical equation that indicates the relationship among the OSI and the significant process variables is given below:

$$OSI = 8.68 - 1.34\ X_3 + 1.32X_3^2 + 0.24X_2X_3 \quad (6)$$

Ethanol concentration showed significant linear and quadratic impact, while irradiation time and temperature did not have any significant linear or quadratic impacts on OSI (P>0.05). Model indicated that significant interaction effect was observed only between ethanol concentration and temperature. As seen in Fig. 1E, the OSI decreased with decreasing of ethanol concentration from 100 to 50%, and then it increased with further increase of water proportion through different extraction temperatures. Thus, the highest level of OSI (11.94 h) was obtained with pure water at 25°C for 50 min.

Solvent polarity is the most important parameter for extraction of polyphenols compared to other extraction variables (Wang et al. 2008). Assessment of extracts in the polar environment such as DPPH and FRAP tests revealed than samples extracted by ethanol-water 50% were the highest antioxidant activities compared to pure ethanol and water. Whereas, samples in Rancimat assay showed different behavior and extracts extracted with water had the maximum of OSI. This reason can be explained by presence of short chain polyphenols with high thermal stability in water. Our results water concurred with Rezaie *et al*. (2015) that reported water extract of Bene hull had more OSI compared to ethanolic extract.

Optimization of UAE Conditions

The optimization of independent factors for ultrasound-assisted extraction (UAE) of Bene hull bioactive compounds were estimated through considering the polynomial models and surface plots. The optimized process conditions were 26.91 min sonication time, 50.42°C temperature and 55.84% aqueous ethanol with desirability of 0.903. The maximum TPC, DPPH$^{\bullet}$ scavenging activity, FRAP and OSI predicted by RSM were 304.47 mg GAE/g, 72.47%, 54.04 mmol/100g and 8.55 h, respectively. Under these optimal conditions the experimental values for TPC, DPPH, FRAP and OSI were 305.62 mg GAE/g, 74.26%, 55.12 mmol/100g and 8.82 h, which were very close to the predicted values by RSM. These results were in agreement by (Kadam *et al*. 2015, Rodríguez-Pérez et al. 2015, Saikia *et al.* 2015) that reported use of ultrasound heat at 45-60°C can increase extraction efficiency of bioactive compounds in shortest time. Because, thermal effects and created cavitation in the liquid phase during sonication lead to cell wall damage, reduction of particle size and subsequently increase of process efficiency (Xu and Pan *et al*. 2013).

HPLC Analysis of the Extracted Polyphenols

The high performance liquid chromatography analysis was performed for identification the major polyphenols in extracted sample under optimal UAE conditions (Fig. 2). Five polyphenols were found in Bene hull extract containing gallic acid, chlorogenic acid, caffeic acid, epicatechin and sinapic acid with retention times 5.18, 15.73, 16.9, 20.41, 23.56 min, respectively. Among the five identified and quantified polyphenols, gallic acid was the major polyphenols in Bene hull extract (1236.65 ppm) and the content of epicatechin, caffeic acid, chlorogenic acid and sinapic acid were 189.39, 64.56, 46.20 and 31.48 ppm, respectively. Therefore, the high level of antioxidant potential of Bene hull is probably due to the presence of large amount of gallic acid. In recent studies, the presence of luteolin, gallic acid, quercetin 3-rutinoside, 2″-O-

galloylisoquercitrin, epicatechin, flavanomarein, ethyl vanillin, and apigenin 7-glucoside were confirmed in Bene hull extract obtained by maceration and subcritical water methods (Shaddel et al. 2014, Rezaie *et al.* 2016). Although, chlorogenic acid, caffeic acid and sinapic acid was not identified in these published works.

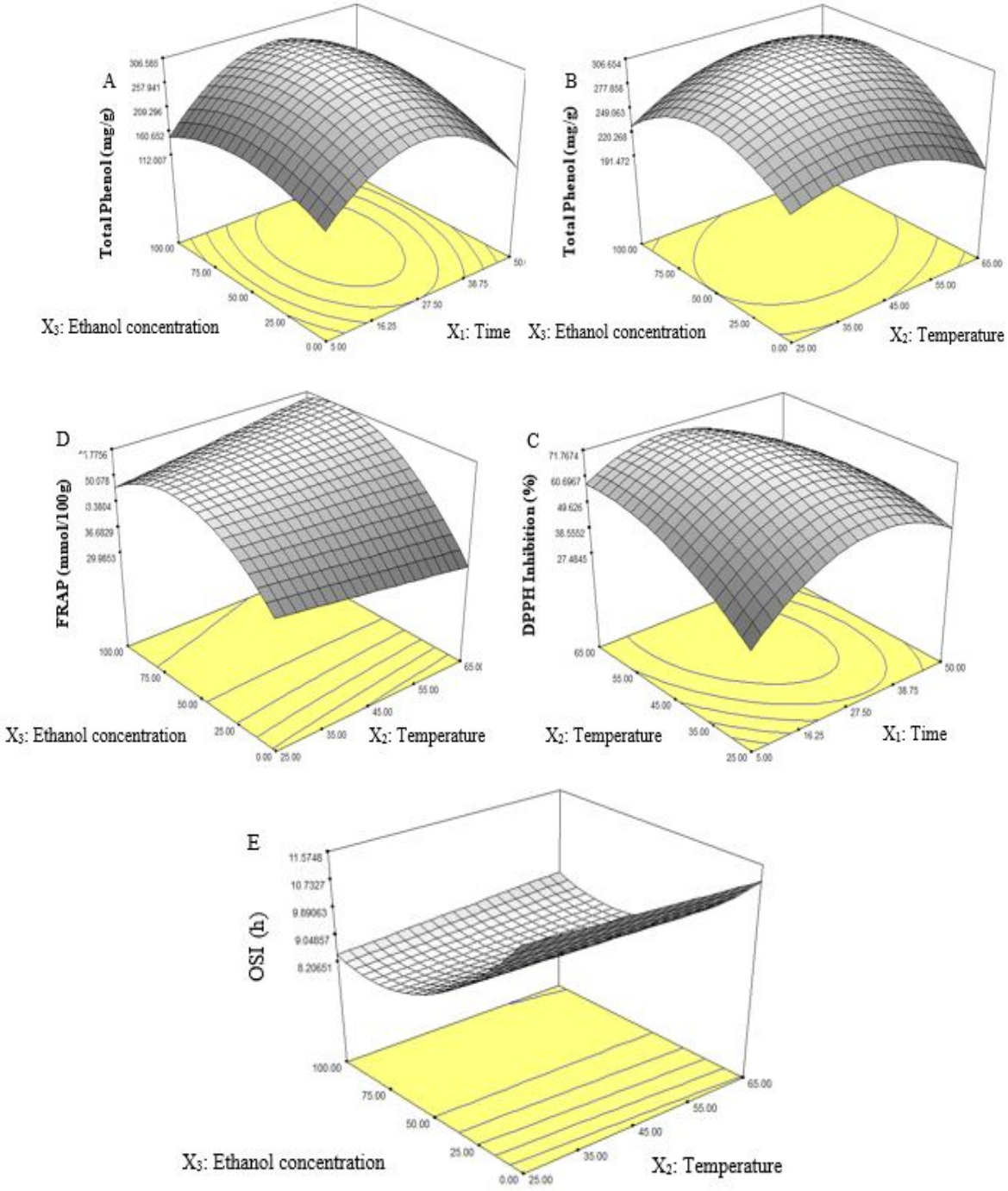

Fig. 1. Response surface plots showing the effect of interaction between independent variables on TPC (A, B), DPPH (C), FRAP (D) and OSI (E) values.

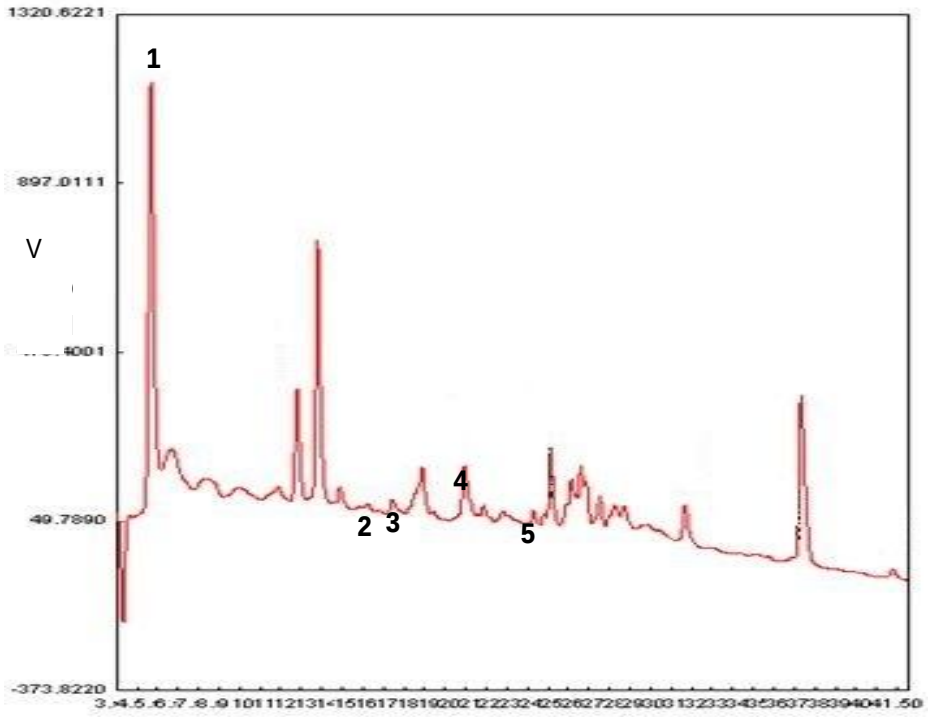

Fig. 2. HPLC chromatogram of phenolic compounds present in bene hull extract. Compounds were identified as follows: (1) gallic acid; (2) chlorogenic acid; (3) caffeic acid; (4) epicatechin; (5) sinapic acid.

Conclusions

Response surface analysis by central composite rotatable design was found as an excellent statistical method for evaluating the effects of extraction variables on total polyphenols and biological activity of Bene hull extract. The experimental values were fitted with second-order polynomial equations. The optimum operating conditions for ultrasound-assisted extraction were 55.84% aqueous ethanol at 50.42°C for 26.91 min based on maximum total polyphenols and antioxidant activity. The TPC, DPPH, FRAP and OSI of optimal extract were 304.47 mg GAE/g, 72.47%, 54.04 mmol/100g and 8.55 h, respectively. Thus, the amount of ethanol in aqueous solvent was important factor for extraction of polyphenols. In addition, HPLC analysis allowed the detection and quantification of five phenolic compounds caffeic acid, chlorogenic acid, gallic acid, epicatechin and sinapic acid in optimal extract

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تأثیر استخراج با فراصوت بر فعالیت بیولوژیکی عصاره پوست بنه (*Pistacia Atlantica* Subsp. *Mutica*): بررسی شرایط بهینه و فعالیت آنتی‌اکسیدانی

مجتبی دلفانیان[1]- محمدحسین حدادخداپرست[2*]- سید محمدعلی رضوی[2]- رضا اسماعیل‌زاده کناری[3]

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چکیده

در این تحقیق از طرح مرکب مرکزی محوری قابل چرخش در روش سطح پاسخ برای بهینه‌یابی شرایط استخراج با فراصوت ترکیبات پلی‌فنلی پوست بنه (*Pistacia atlantica* subsp. *Mutica*) استفاده شد. پارامترهای زمان، دما و نسبت حلال اتانول/ آب از پارامترهای مستقل بررسی شده برای بهینه‌یابی شرایط استخراج بودند. میزان ترکیبات پلی‌فنلی تام و قدرت آنتی‌اکسیدانی عصاره‌ها از نظر قدرت احیاکنندگی آهن (FRAP)، جذب رادیکال‌های آزاد DPPH و شاخص پایداری اکسایشی (OSI) تعیین شد. داده‌های حاصل با معادلات درجه دوم با اثرات خطی، درجه دوم و متقابل فاکتورهای فرآیند به خوبی سازگار بود. شرایط بهینه استخراج در زمان 26/91 دقیقه، دمای 50/42 درجه سانتی‌گراد و با نسبت اتانول 55/84 درصد ایجاد شد. میزان ترکیبات پلی‌فنلی تام و قدرت جذب رادیکال‌های آزاد DPPH، قدرت احیاکنندگی آهن (FRAP)، و شاخص پایداری اکسایشی عصاره استخراجی در شرایط بهینه به ترتیب 304/47 میلی‌گرم گالیک اسید بر گرم، 72/47 درصد، 54/04 میلی‌مول بر 100 گرم و 8/55 ساعت بود. آنالیز عصاره بهینه با کروماتوگرافی مایع با عملکرد بالا (HPLC) حضور اپی‌کاتچین، کلروژنیک اسید، سیناپیک اسید، کافئیک اسید و گالیک اسید را شناسایی کرد.

واژه‌های کلیدی: پوست بنه، فعالیت آنتی‌اکسیدانی، پلی‌فنل، روش سطح پاسخ، استخراج با فراصوت

1 و2- به‌ترتیب دانشجوی دکترا و استاد، گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد
3- دانشیار، گروه علوم و صنایع غذایی دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران
(*- نویسنده مسئول : Email: khodaparast@um.ac.ir)