

Comparative study of extraction methods for pistachio hull antioxidants by multiple assays

Reza Tabaraki* and Farzaneh Ghadiri

Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran

Article history:

Received: 28/Feb/2015

Received in revised form: 12/Sep/2015

Accepted: 17/Jun/2016

Abstract

In recent years, the use of natural antioxidants extracted from agricultural and industrial by-products has been increased because of sustainability, high and stable antioxidant activity, absence of toxicity. These extracts can be used as substitute of synthetic antioxidants for food products, color and oxidative stabilization. In this study, experimental design and response surface methodology (RSM) were used to optimize experimental variables such as sample weight (g), irradiation power and time (s) in microwave-assisted extraction (MAE) of antioxidants from pistachio hull. Effect of sample weight was found to be significant on total phenolic content (TPC), ferric reducing antioxidant power (FRAP), scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and extraction yields. The optimal conditions were water as solvent, particle size of 0.25 mm, microwave power 600 W, sample weight 0.2 g, irradiation time of 150 s. Finally, comparison of extraction methods was shown that MAE method gave better results than ultrasonic-assisted extraction (UAE) and conventional methods with the optimum operating conditions like time and energy consumption.

Keywords: Extraction, Pistachio hull, Total phenolic content, Ferric reducing antioxidant power, DPPH, Microwave.

1. Introduction

The human body is exposed to a large variety of reactive species from both endogenous and exogenous sources. To protect the cells and organs against free radicals, biological systems have evolved an antioxidant protection system. These antioxidants therefore constitute the body's first line of defence system. When the availability of antioxidants is limited, cell damages and food oxidation occurs. Cell damage caused by free radicals has been implicated in the pathogenesis of at least 50 diseases conditions such as atherosclerosis, brain disfunction and cancer [1].

Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are largely used in food industry and included in human diet. However, in recent years the use of natural antioxidants has been promoted because of concerns regarding the safety of synthetic ones [2]. For wider industrial applications, natural antioxidants have to meet important requirements, such as sustainability, high and stable antioxidant activity, absence of toxicity and others. There has been an increasing interest in extraction of antioxidants from agricultural and industrial by-products [3].

Pistachio is considered as a very important nutritional product (100 grams of edible pistachio contains about 600 calories, in which 53% fat, 21% protein, 18% carbohydrates, 2.2% fiber and no cholesterol). Pistachio is also rich in vitamins such as B₁, B₂, C, and E. Pistachio (*Pistachio vera*) hull (PH) is by-product of dehulling of pistachio nuts after harvesting. According to Food and Agricultural Organization (FAO), total production of pistachio in Iran was about 446,647 ton (about 49% of the world's production) in 2010 and Iran is the largest exporter in the world. Pistachio hull is a good source of natural phenolics and antioxidants [4]. Phenolics content and antioxidant activity of pistachio hull are more than those of the skin and nut [5]. Inhibitory effects of a flavonoid-rich extract of *Pistacia vera* hull on growth and acid production of bacteria involved in dental plaque were investigated (6). Antioxidant, antimicrobial and antimutagenicity

activities of pistachio (Ahmadaghaei variety) green hull extracts (crude and purified extracts) were also studied [7, 8].

Recent years has seen an increasing demand for new extraction techniques enabling automation, shortening extraction times and reduction of organic solvent consumption. These extraction techniques are microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SFE). Conventional extraction methods have been associated with high solvent requirements, longer extraction times and increased risk of degradation of thermo-labile constituents. Up-to now, different extraction techniques have been reported for the extraction of polyphenols and antioxidants from pistachio hull such as supercritical fluid extraction and ultrasonic-assisted extraction [4]. Disadvantages of supercritical fluid extraction are higher cost of the equipment and the blockage in the systems as a result of the presence of water in the sample. Published studies under ultrasound conditions indicate increased yield or extraction rate as well as reduction in extraction time. Microwave-assisted extraction (MAE) can also increase yield in shorter times. Unlike classical conductive heating methods, microwaves heat the whole sample simultaneously and homogeneously. Owing to their electromagnetic nature, microwaves electric field causes heating via two simultaneous mechanisms, namely, dipolar rotation and ionic conduction.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process. Unlike the conventional empirical method, RSM can generate a mathematical model, and take into account the possible inter relationship among the test variables while minimizing the number of experiments [9].

The aim of this study was to optimize microwave-assisted extraction variables such as microwave power, time and amount of sample for extraction of antioxidants from pistachio hulls by response surface methodology. Finally, microwave-assisted, ultrasonic-assisted extraction and conventional extraction methods were compared.

2. Material and methods

2.1. Plant material

Raw pistachio was obtained from the Semnan, Iran. Fruits were manually dehulled and collected hulls were then rinsed with distilled water. The hulls were dried in an oven (Memmert, GmbH+ Co. KG, DIN 40050, Germany) with air circulation at 40 °C, and they were finely ground in a laboratory grinder (Pars Co., Iran). The ground sample was fractioned by a series of sieves (0.5, 0.25, 0.18 and 0.106 mm, Damavand Co., Iran) to obtain the particle size distribution. The dry sample was then stored at -20 °C.

2.2. Chemicals

2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu reagent and gallic acid were purchased from Merck. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich.

2.3. Microwave-assisted extraction procedure

Microwave-assisted extraction (MAE) was performed in an microwave oven (Samsung, Model CE3280EB) with maximum power of 900 W. Different amounts of powdered sample (0.1 g, 0.3 g, 0.5 g) were mixed with 10 ml distilled water. The mixture was placed in the center of a microwave oven, containing a circular, 360° rotating carousel with different durations of exposure: 50, 100, 150 s and with different powers: 300, 600 and 900 W. The suspensions were irradiated with microwaves as follows: 2s power on (for heating, temperature about 50-60 °C) and 15 s power off (for cooling) and so on to the pre-set extraction time. Super-boiling of the solution did not occur. At the end of extraction, the glass vessel was allowed to cool down to room temperature, centrifuged at 4500 rpm for 15 min and then filtered through a filter paper. Water was evaporated from the remaining filtrate fraction using a

rotary evaporation at 40 °C under vacuum to dryness and the yield of extraction was determined.

2.4. Ultrasonic-assisted extraction procedure

Ultrasonic-assisted extraction was performed in an ultrasonic bath RK103H (BANDELIN SONOREX, Germany) with a maximum capacity of 4 L (35 KHZ, 140 W). Pistachio hull powder (0.2 g) was sonicated in the solvent (10 ml) for different times at required temperature. For the ultrasound-assisted extraction (UAE), the material was set in a water bath and the temperature was measured by a thermometer. After the extraction, the pistachio hull extract was centrifuged at 4500 rpm for 15 min, and then the solution was filtered. The extracts were concentrated by rotary evaporation at 45 °C under vacuum to dryness and the yield of extraction was determined.

2.5. Conventional extraction method

In this stage, dried sample (0.2 g) was subjected to extraction by adding 10 mL distilled water in a vessel with a magnetic stirrer. Then the samples were heated in a water bath with constant temperature of 30 °C at different times (15, 45, 90, 120 and 180 min).

2.6. Optimization of solvent type and particle size

At the beginning of this study, effect of solvent type was investigated. It is evident that the recovery of antioxidant compounds from sample matrix is dependent on the extracting solvent. In this study, several extraction solvents such as water, methanol, ethanol and ethyl acetate were used due to the wide range of polarity of antioxidants under the same extraction conditions (50:1(v:m), 300 W irradiation power, particle size 0.25 mm and 50 s irradiation time). Results were expressed as means \pm standard deviations of triplicate measurements Pistachio hull powder was passed through four different standard-size sieves (pore sizes of 0.5, 0.25 and 0.18, 0.106 mm). Microwave-assisted extraction of antioxidants was performed at 300 W irradiation power, 50 second irradiation time and solvent to solid ratio 50:1 with water as solvent.

2.7. Total phenolic content (TPC)

The total phenolic content of the pistachio hull extracts was determined using the Folin–Ciocalteu reagent [10]. Forty microliters of 10 times diluted pistachio hull extract solution were mixed with 1.8 mL of 1:10 diluted FC reagent. The mixture was kept for 5 min at room temperature, then 1.2 mL of (7.5% w/v) sodium carbonate solution were added. The solution was mixed and allowed to stand for 1 h at room temperature. Finally, the absorbance was measured at 765 nm, using a UV–visible spectrophotometer. A calibration curve was prepared using standard solutions of gallic acid. The results of total phenolic content were expressed as mg gallic acid equivalents per g dry weight of pistachio hull.

2.8. FRAP method

The FRAP assay was carried out according to the procedure of Benzie and Strain [11] with slight modification. In brief, the FRAP reagent was prepared from sodium acetate buffer (300 mM, pH=3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM FeCl₃ solution in proportions of 10:1:1 (v/v), respectively. The fresh FRAP reagent was prepared fresh daily and warmed to 37°C in a water bath prior to use. Fifty microliters of 1:10 diluted sample were added to 1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. All measurements were carried out in triplicate. The standard curve was constructed using FeSO₄ solution. The results were expressed as μmol Fe (II)/g dry weight of pistachio hull.

2.9. DPPH radical-scavenging activity

DPPH radical-scavenging activity of pistachio hull extract was determined according to the method reported by Brand-Williams [12] with some modification. An aliquot of 0.5 mL of 1:10 diluted sample solution was mixed with 2.5 mL of a 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a blank, using a UV–Vis spectrophotometer. Results were expressed as percentage of inhibition of

the DPPH radical. Percentage of inhibition was calculated according to the following equation:

$$\% \text{ Inhibition of DPPH} = [(A_{\text{DPPH}} - A_s) / A_{\text{DPPH}}] \times 100$$

(Equation 1)

where A_{DPPH} is the absorbance of DPPH solution without extracts.

2.10. Experimental design and statistical analysis

Response surface methodology was applied to determine the optimized conditions using microwave technique for the extraction of natural antioxidants from pistachio hull. Statistical analysis was performed using the Minitab 16 (Minitab Inc., State College, PA, USA) software and fitted to a second-order polynomial regression model containing the coefficient of linear, quadratic and interaction terms. Central composite design (CCD) was used to investigate the effects of three independent variables (microwave power, irradiation time and sample weight) at three levels on the dependent variables (TPC, FRAP, DPPH and yield). Each variable was coded at three levels, -1, 0 and +1. The quadratic model for each response was as follows:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} X_i X_j$$

(Equation 2)

where β_0 , β_i , β_{ii} , β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are coded value of the independent variables while k equals to the number of the tested factors ($k = 3$).

All the analysis was carried out in triplicates and the experimental results were expressed as means \pm SD. An analysis of variance (ANOVA) with 95% confidence level was then carried out for each response variable in order to test the model significance and suitability. The significances of all terms in the polynomial were statistically analyzed by computing the F -value at a probability (p) of 0.001, 0.01 or 0.05. The absence of any lack of fit ($p > 0.05$) also strengthened the reliability of all models. The models were used for the construction of three dimensional response surface plots to predict the relationship between independent and dependent variables.

3. Results and discussion

3.1. Effect of solvent type

It is evident that the recovery of antioxidant compounds from sample matrix is dependent on the extracting solvent. In this study, several extraction solvents such as water, methanol, ethanol and ethyl acetate were used due to the wide range of polarity of (Fig. 1A). The results showed that extraction with water exhibited the highest TPC, FRAP and extraction yield. The highest DPPH value was obtained from methanol as extraction solvent. The ANOVA was indicated that differences between solvents on the extraction of antioxidants were significant except for ethanol and ethyl acetate in FRAP (Fig. 1A). Solvents with high dielectric constants (e.g. water) can absorb more microwave energy and the polarity of the solvent is very important in microwave extraction [13]. More polar phenolic compounds may be extracted according to "like dissolves like" principle.

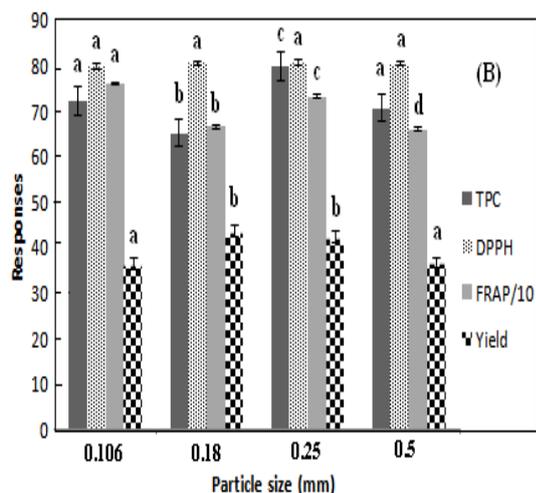
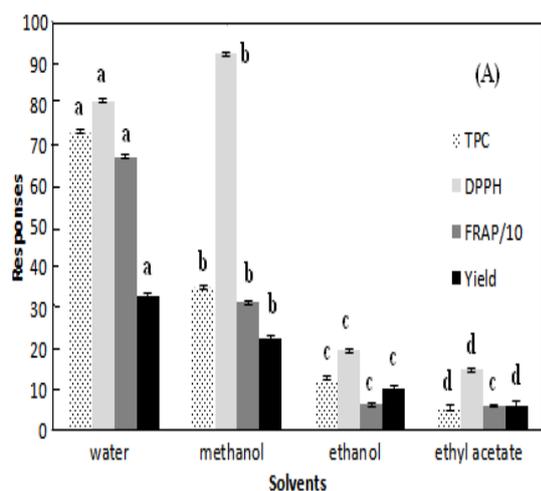


Fig. 1 Effect of (A) solvent type (B) particle size on the TPC, DPPH, FRAP and extraction yield over a 50 second irradiation time at 300 W. TPC: mg gallic acid equivalents per g dry weight; DPPH: % inhibition; FRAP: $\mu\text{mol Fe}^{2+}/\text{gdw}$. Each observation is a mean \pm SD of three replicate experiments.

Environmentally benign and non-toxic food grade solvents like water and ethanol are recommended by the US Food and Drug Administration for extraction purposes [14]. So water was chosen as the extraction solvent for the next experiments.

3.2. Effect of particle size

Effect of particle size on microwave-assisted extraction of antioxidants was shown in Fig. 1B. The results showed that extractions with 0.25 mm particles exhibited the highest TPC. The highest FRAP values were obtained with 0.18 mm particles. The ANOVA was indicated that differences between particle sizes on the extraction of antioxidants were not significant for DPPH. The ANOVA was also indicated that differences between 0.18 and 0.25 mm particle sizes on extraction yields were not significant. Therefore, particle size of 0.25 mm was used for next experiments. The increase of the extraction yield for the small particles is due to larger surface area per mass unit. In addition, the migration rate of the analyte through the pores of the solid matrix is also increased with the decrease in particle size [15].

3.3. Modeling of the extraction process

The responses (total phenolic content, antioxidant activities and yield) of each run of the experimental design were presented in Table 1. The coded values of independent variables for each experiment are also presented. Total phenolic content of pistachio hull extracts varied from 49.2 to 74.6 mg GA/g dry sample. FRAP and scavenging of DPPH radical assays were used to determine the antioxidant activity of the extracts.

As shown in Table 1, activity values varied from 282.1 to 659 $\mu\text{mol Fe}^{2+}/\text{g}$ of dry sample, 69.5% to 81.2% for FRAP and DPPH assays, respectively. Extraction

yields ranged from 40 to 55.2%. ANOVA was used to estimate

the statistical significance of the factors and interactions between them. Regression coefficient and analysis of variance of the second-order polynomial models for total phenolic content, antioxidant activity of pistachio hull extracts and yield are summarized in Table 2. As shown, the regression parameters of the surface response analysis of the models, the linear, quadratic and interaction terms have significant effects. The large values of the R^2 indicated that the models adequately represent the experimental results. The absence of any lack of fit ($p > 0.05$) also strengthened the reliability of all models. The models were used for the construction of three dimensional response surface plots to predict the relationship between independent and dependent variables.

3.4. Effect of process variables

Total phenolic contents of pistachio hull extracts obtained by microwave-assisted extraction are shown in Table 1. Regression analysis was performed on the experimental data and the coefficients of model were evaluated for significance. Sample mass demonstrated a pronounced influence on TPC in linear, quadratic and cross product manner (Table 2). Equation (3) shows the relationship between sample mass, microwave power and irradiation time for the extraction of total phenolic compounds:

$$Y_1 \text{ (mg GA/gdw)} = 39.65 + 163.86 \times M + 0.34 \times 10^{-1} \times P - 0.31 \times t - 240.60 \times M^2 - 0.01 \times 10^{-2} \times P^2 + 0.02 \times 10^{-1} \times t^2 + 0.43 \times 10^{-1} \times M \times P - 0.20 \times M \times t - 0.01 \times 10^{-2} \times P \times t \text{ (Equation 3)}$$

where Y_1 represents total phenolis in pistachio hull extract and M, P and t are sample weight, microwave power and time, respectively.

Three-dimensional response surface plots (Fig. 2A) illustrate the relationship between total phenolic content of pistachio hull extract and experimental variables. This graphical representation is an n -dimensional surface in the $(n+1)$ -dimensional space. These plots present the response in function of two factors and keep the other variable constant at its middle level. Analysis of the experimental results

showed that the sample weight had the greatest effect on TPC. The effect of microwave power and sample weight on TPC at constant time appeared as a saddled shape. As shown, TPC gradually mounted up with the increase of sample weight and microwave power, and achieved optimum value at about 0.3g, before it began to decrease. Longer extraction time had positive effects on the TPC.

The analytical results of antioxidant activities (DPPH and FRAP) of pistachio hull extracts are shown in Table 2. The results of regression analysis indicated that the main extraction parameter for antioxidant compounds from pistachio hull was sample weight. The relationship between the antioxidant activities and extraction variables are shown in Figures 2B and 2C for DPPH and FRAP, respectively. Antioxidant activities were affected by linear and quadratic terms of sample weight. The models for antioxidant activities are represented in equations (4) and (5):

$$Y_2 \text{ (% inhibition)} = 67.32 + 52.83 \times M - 0.01 \times 10^{-1} \times P + 0.12 \times t - 107.89 \times M^2 - 0.01 \times 10^{-2} \times P^2 - 0.01 \times 10^{-1} \times t^2 + 0.01 \times 10^{-1} \times M \times P + 0.15 \times 10^{-1} \times M \times t + 0.01 \times 10^{-2} \times P \times t \text{ (Equation 4)}$$

$$Y_3 \text{ (}\mu\text{mol Fe}^{2+}\text{/g dw)} = 239.52 + 1599.52 \times M + 0.93 \times P - 3.11 \times t - 3464.61 \times M^2 - 0.01 \times 10^{-1} \times P^2 + 0.02 \times t^2 - 0.01 \times M \times P + 1.11 \times M \times t - 0.01 \times 10^{-1} \times P \times t \text{ (Equation 5)}$$

where Y_2 and Y_3 represent scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) of pistachio hull extracts.

Table 1. Central composite design of three variables with their observed responses.

Exp. No	X ₁	X ₂	X ₃	Sample mass (g)	Power (W)	Time (sec)	TPC (mgGA/gdw)	DPPH (%)	FRAP (μmolFe ²⁺ /gdw)	Yield (%)
1	1	1	1	0.5	900	150	65.6	72.2	389.6	40.5
2	1	-1	1	0.5	300	150	59.7	69.6	418.4	40.0
3	-1	-1	-1	0.1	300	50	49.4	75.2	475.0	44.4
4	0	-1	0	0.3	300	100	63.3	81.2	529.0	43.2
5	0	0	0	0.3	600	100	61.5	78.5	590.8	41.7
6	-1	1	1	0.1	900	150	53.1	75.4	540.0	52.8
7	1	-1	-1	0.5	300	50	59.7	71.2	282.1	43.6
8	0	0	-1	0.3	600	50	69.6	78.7	631.0	44.2
9	0	0	1	0.3	600	150	74.6	78.1	659.0	46.8
10	-1	1	-1	0.1	900	50	51.6	74.9	520.0	50.8
11	-1	0	0	0.1	600	100	49.2	77.1	545.0	51.2
12	1	1	-1	0.5	900	50	67.0	69.5	383.5	42.8
13	1	0	0	0.5	600	100	63.9	74.9	369.2	41.0
14	-1	-1	1	0.1	300	150	62.6	74.6	508.8	55.2
15	0	1	0	0.3	900	100	64.0	78.8	549.0	42.5
16	0	0	0	0.3	600	100	64.3	77.6	552.3	40.9

TPC: total phenolic content; DPPH: scavenging activity of 2, 2-diphenyl-1-picrylhydrazyl radical; FRAP: Ferric reducing antioxidant power.

Table 2. Regression coefficients of predicted polynomial models

Coefficient	Responses			
	TPC	DPPH	FRAP	Yield
β_0	39.65**	67.32***	239.52*	45.04***
β_1	163.86**	52.83*	1599.52***	-31.82
β_2	0.34×10^{-1}	-0.01×10^{-1}	0.93**	0.02
β_3	-0.31	0.12	-3.11	-0.24×10^{-1}
β_{11}	-240.60**	-107.89**	-3464.61***	62.20
β_{22}	-0.01×10^{-2}	-0.01×10^{-2}	-0.01×10^{-1} *	-0.01×10^{-3}
β_{33}	0.02×10^{-1} *	-0.01×10^{-1}	0.02*	0.08×10^{-2}
β_{12}	0.43×10^{-1} *	0.01×10^{-1}	-0.01	-0.90×10^{-2}
β_{13}	-0.20	0.15×10^{-1}	1.11	-0.23*
β_{23}	-0.01×10^{-2}	0.01×10^{-2}	-0.01×10^{-1}	-0.01×10^{-2}
Model	***	*	***	**
Linear	***	*	**	ns
Quadratic	***	**	***	ns
Cross-product	ns	ns	ns	ns
Lack of fit	ns	ns	ns	ns
R ²	0.94	0.92	0.97	0.93

^a Polynomial model $Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_i X_i^2 + \sum_{i=1}^{k-1} \sum_j^k \beta_{ij} X_i X_j$

where β_0 is the constant coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient.

^b *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$.

^c ns, not significant ($p > 0.05$).

TPC: total phenolic content; DPPH: scavenging activity of 2, 2-diphenyl-1-picrylhydrazyl radical; FRAP: Ferric reducing antioxidant power.

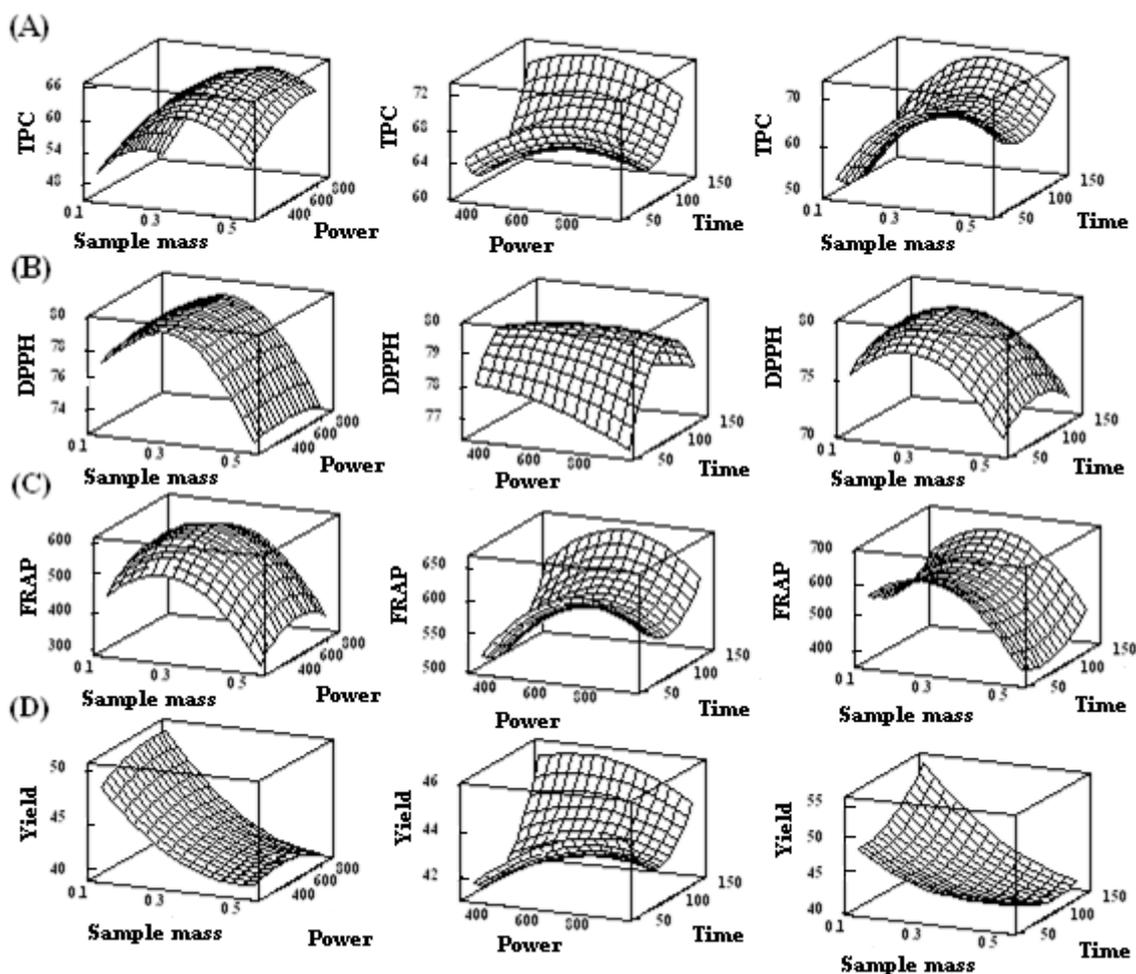


Fig. 2. Response surface plots showing the effects of sample mass/power, power/time and sample mass/time on (A) total phenolic content (TPC), (B) scavenging activity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (C) Ferric reducing antioxidant power (FRAP), (D) extraction yield.

The effect of microwave power and time on FRAP at constant sample mass appeared as saddled shape (Fig. 2C). Antioxidant activities gradually mounted up with the increase of sample weight and microwave power, and achieved optimum values at about (0.3 g) for DPPH and FRAP, before it began to decrease (Figs. 2B and 2C). As shown in response surface plots for the effect of time, antioxidant activities achieved optimum values for DPPH and FRAP values at about 100 and 150 seconds, respectively.

The yields of extractions are presented in Table 1. Solvent was removed from the extracts by evaporation under vacuum at 45°C by a rotary evaporator. The regression analysis of the data showed that the extraction yield was significantly affected by the interaction term of sample weight and time. The

relationship of the extraction yield and process variables is depicted in Fig. 2D. Equation (6) shows relationship between sample weight, microwave power, time and extraction yield:

$$Y_4 (\%) = 45.04 - 31.82 \times M + 0.02 \times P - 0.24 \times 10^{-1} \times t + 62.20 \times M^2 - 0.01 \times 10^{-3} \times P^2 + 0.08 \times 10^{-2} \times t^2 - 0.09 \times 10^{-2} \times M \times P - 0.23M \times t - 0.01 \times 10^{-2} \times P \times t \quad (\text{Equation 6})$$

where Y_4 is the extraction yield (%) and M , P and T are sample weight, microwave power and extraction time, respectively.

The relationship between the extraction yield and process variables are shown in Fig. 2D. Maximum of extraction yield was obtained when sample weight, power and time were 0.1 g, 300 W and 150 second, respectively.

3.5. Optimal conditions

Optimum MAE conditions for the yield of extraction (0.1 g, 300W and 150 sec irradiation time), TPC (0.3 g, 300W and 50 sec), DPPH (0.3 g, 300W and 80 sec) and (0.3 g, 300W and 150 sec) were calculated from RSM models. Predicted and experimental values in these conditions were (54.6 and 55.0%), (57.2 and 60.3 mg GAE/gdw), (70.2 and 73.5%) and (584.1 and 614.3 $\mu\text{mol Fe}^{2+}/\text{g}$ of dry sample) for yield, TPC, DPPH and FRAP, respectively. The extract can be used as substitute of synthetic antioxidants for food products, color and oxidative stabilization. Therefore, the highest yield is recommended for industrial applications. The optimal conditions for this aim were water as solvent, particle size of 0.25 mm, microwave power 300 W, sample weight 0.1 g, irradiation time of 150 s. Each antioxidant assay (TPC, FRAP, DPPH ...) only provides an estimate of antioxidant capacity that is subjective to its conditions, reagents and different classes of antioxidants. Therefore, the use of different antioxidant assays help to identify variations in the response of the compounds extracted from the samples. These natural antioxidant compounds can be separated by HPLC from the extract. The predicted results matched well with the experimental results obtained using optimum extraction conditions which validated the RSM models.

The multi-response optimization for extraction was done by desirability function approach. The maximization of these four responses is of practical importance since they might conflict with each other. Therefore, it is necessary to find out the optimal point as a compromise for the maximal FRAP, TPC, DPPH and yield. The individual desirability (d_i) for Y_1 , Y_2 , Y_3 and Y_4 were calculated by one side transformation. The individual desirability's were then used to calculate overall desirability (D) of the optimization. The scale in the range of 0.0 (undesirable) to 1.0 (very desirable) is used to obtain a global function (D) that should be maximized according to efficient selection of designed variables. The overall desirability of optimization was found to be $D=0.76$. At this D value, optimum values

of the selected variables were microwave power 600 W, sample weight 0.2 g, irradiation time of 150 s which responded for $Y_1 = 69.7$ mg GAE/gdw, $Y_3=77.6\%$, $Y_2=659 \mu\text{mol Fe}^{2+}/\text{g}$ of dry sample and $Y_4= 49.4\%$.

Table 3. Comparison of microwave-assisted and ultrasonic-assisted extraction and conventional extraction of antioxidants from pistachio hulls in optimum conditions for each method

Method	TPC (mg GAE/gdw)	FRAP ($\mu\text{mol Fe}^{2+}/\text{gdw}$)	DPPH (%)	Yield (%)
Conventional	45.9	380.4	80.5	34.4
UAE	58.1	401.3	84.2	41.1
MAE	62.6	508.8	74.6	55.2

3.6. Comparison of three extraction methods

Effects of solvent type, solvent to solid ratio, particle size, temperature and time on the TPC, DPPH, FRAP and extraction yield in ultrasonic-assisted extraction of antioxidants from pistachio hull were also studied. Results were shown as means \pm standard deviations of triplicate measurements in Figs. 3A-3E. As shown, the optimal conditions for ultrasonic-assisted extraction were water as solvent, solvent to solid ratio of 50:1, particle size of 0.25 mm, temperature of 30 °C and time of 45 min.

Conventional extraction was performed with water as solvent, solvent to solid ratio of 50:1, particle size of 0.25 mm, temperature of 30 °C. Effect of extraction time was shown in Fig. 3F. Optimal extraction time was 90 min.

Experimental data of antioxidant extraction from pistachio hull by MAE, UAE and conventional methods at optimal conditions of each method were presented in Table 3. MAE method showed the best effect on the extraction of antioxidants from pistachio hull. The shortest process time was for MAE (150 s irradiation time, total time of 21 min) with respect to UAE (45 min) and conventional methods (90 min). The consumed energy was equal to the power multiply by the exposure time. The energy consumption was (300 W \times 2.5 min) and (140 W and 45 min) for MAE and UAE, respectively. Thus, MAE method gave better

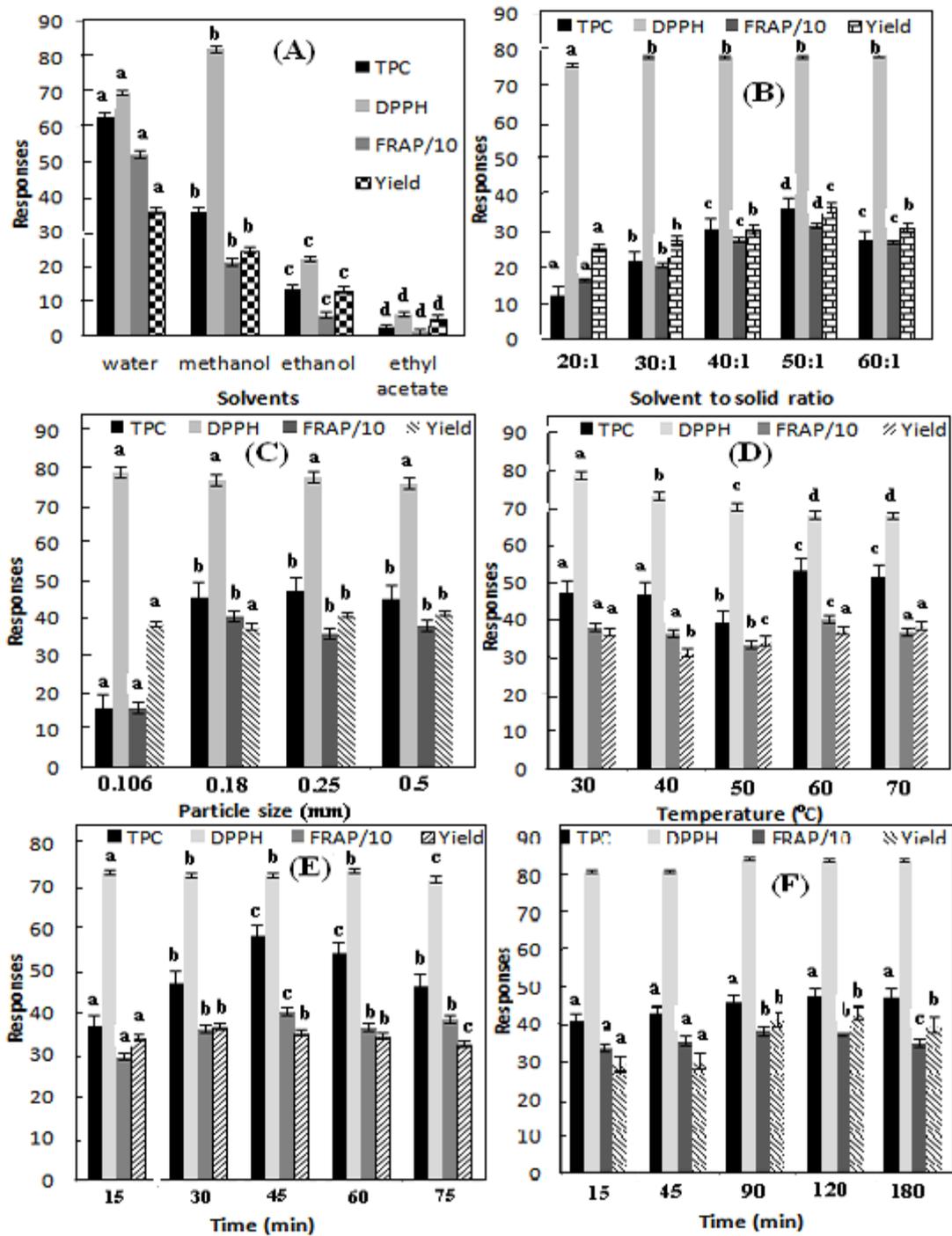


Fig. 3. Effect of (A) solvent type, (B) solvent to solid ratio, (C) particle size, (D) temperature and (E) time on the TPC, DPPH, FRAP and extraction yield in ultrasonic assisted extraction. (F) effect of time in conventional extraction; TPC (mg gallic acid equivalents/gdw); DPPH: (% inhibition); FRAP ($\mu\text{mol Fe}^{2+}/\text{gdw}$); Yield (%); Each observation is a mean \pm SD of three replicate experiments.

results than UAE and conventional methods with the optimum operating conditions like time and energy consumption.

Theoretically, microwave radiation loosens the cell wall matrix and thereby the skin tissues are rapidly and extensively opened up by the microwave. This will lead to increased interaction between extracting agent and source material in extraction process. As a result, permeation of the extracting agent will be increased. It leads to effective increase in the yield of extraction [16, 17].

References

- [1] T. Finkel and N.J. Holbrook, *Nature*, **408** (2000) 239–247.
- [2] M. Mohadjerani and K. Pakzad, *Journal of Applied Chemistry*, **7** (2013) 45–48.
- [3] N. Balasundram, K. Sundram and S. Samman, *Food Chemistry*, **99** (2006) 191–203.
- [4] A. H. Goli, M. Barzegar and M. Sahari, *Food Chemistry*, **92** (2005) 521–525.
- [5] M. Behgar, S. Ghasemi, A. Naserian, A. Borzoie and H. J. Fatollahi, *Journal of Radiation Physics and Chemistry*, **80** (2011) 963–967.
- [6] Y. Y. Kamrani, M. Amanlou, B. Esmaeliani, S. M. Bidhendi and M. SahebJamei, *International Journal of Pharmacology*, **3** (2007) 219–226.
- [7] A. Rajaei, M. Barzegar, A. M. Mobarez, M. A. Sahari and Z. H. Esfahani, *Food and Chemical Toxicology*, **48** (2010) 107–112.
- [8] A. Rajaei, M. Barzegar, Z. Hamidi and M. A. Sahari, *Journal of Agricultural Science and Technology*, **12** (2010) 605–615.
- [9] C. Liyana-Pathirana and F. Shahidi, *Food Chemistry*, **93** (2005) 47–56.
- [10] M. Alothman, R. Bhat and A. A. Karim, *Food Chemistry*, **115** (2009) 785–788.
- [11] C. C. Wong, H. B. Li, K. W. Cheng and F. Chen, *Food Chemistry*, **97** (2006) 705–711.
- [12] W. Brand-Williams, M. E. Cuvelier and C. Berset, *LWT – Food Science and Technology*, **28** (1995) 25–30.
- [13] C. Proestos and M. Komaitis, *LWT- Food Science and Technology*, **41** (2008) 652–659.
- [14] D. D. Bartnik, C. M. Mohler and M. Houlihan, United States Patent Application 20060088627, April 27 (2006).
- [15] D. L. Luthria, *Food Chemistry*, **107** (2008) 745–752.
- [16] H. Bagherian, F. Zokaei Ashtiani, A. Fouladitajar and M. Mohtasham, *Journal of Chemical Engineering and Processing*, **50** (2011) 1237–1243.
- [17] G. Spigno and D. M. De Faveri, *Journal of Food Engineering*, **93** (2009) 210–217.

