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Optimization of Extraction Conditions of Phenolic Compounds from Pistachio (*Pistachia vera*) Green Hull through Response Surface Method

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ABSTRACT

Phenolic compounds, especially those of plant origin, constitute an essential part of the human diet, and are of considerable interest due to their antioxidant properties. In this study, Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), as well as Maceration Extraction (ME) methods were applied for phenolic compounds' extraction from pistachio green hull. Response surface methodology was employed to optimize the extraction conditions as regards the yield of the compounds. A Central Composite Design (CCD) was employed to investigate the effects of three independent variables, namely liquid-to-solid ratio (8-20 times), temperature (25-65 °C) and time (5-45 minutes) on the dependent variable (level of total phenolic compounds). The results indicated that within the same extraction time, the extraction yield through UAE was higher than those in ME and MAE methods. Correlation coefficients (R^2) of the models for UAE, MAE and ME methods were 0.95, 0.96 and 0.94, respectively. The optimal conditions for extraction of phenolic compounds from pistachio green hull through ME, UAE, and MAE methods were 20(v/w), 65°C, 45 minutes; 20(v/w), 65°C, 25 minutes; and 20(v/w), 65°C, 45 minutes, respectively. Under optimized conditions the experimental values well agreed with the values predicted by the proposed models.

Keywords: Maceration method, Microwave-assisted extraction, Phenolic compounds, RSM, Ultrasound-assisted extraction.

INTRODUCTION

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plant. Phenolic compounds exhibit a wide range of such physiological properties as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodiolatory effects (Madhavi and Salunkhe, 1995; Pokorny *et al.,* 2001; Vermerris and Nicholson, 2006; Balasundram *et al.,* 2006; Andersen and Markham, 2006).

The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. These compounds could be a major determinant of antioxidant potentials of foods, and a natural source of antioxidants (Balasundram *et al.,* 2006). The research on phenolic compounds has been growing lately because of the increasing worldwide demand for phenolic compounds and their increasing application in food industry (Rodrigues and Pinto, 2006). Extraction is the first step in the isolation of phenolic compounds from plant materials. Such traditional methods as soxhlet extraction, which have been used for many decades, are very time-consuming and require relatively large quantities of solvents (Wang and Weller, 2006). There is an increasing demand for new extraction techniques to

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shorten the extraction time, reduce organic solvent consumption, and to prevent environmental pollution. Novel extraction methods including Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE) and Accelerated Solvent Extraction (ASE) are fast and efficient for extracting chemicals from solid plant matrixes (Wang and Weller, 2006).

Each vegetable material has its own unique properties in terms of phenolic components. Thus, it is important to develop an optimal extraction method. Iran is a leading country in production of pistachio nuts. Total production was about 304,000 tons in 2005 (Anonymous, 2005) with the country being the largest exporter (about 86%) in the world. Recently, it has been established that Pistachio Green Hull (PGH) and its nut possess a high level of phenolic compounds much comparable with those present in already recognized phenolic sources (Chen and Blumberg, 2008; Goli *et al.,* 2005; Yalpani and Tyman, 1983). Also, gallic acid is the most found phenolic compound in PGH with a content of about 80 percent (Vahabzadeh *et al.,* 2004). The potential use of PGH as a source of antioxidant phytochemicals calls for an optimization of the extraction process. The response surface methodology (RSM) not only is all factors at the time of approach allow accounting for possible interaction effects between variables. If adequately used, this powerful tool can provide the optimal conditions that improve a process (Haaland, 1989).

The objective of this study was: to determine the optimal extraction conditions for phenolic compounds through ME, UAE, and MAE and to compare this methods together.

MATERIALS AND METHODS

Plant Materials and Chemicals

Pistachio green hulls (*Ahmadaghaei* variety) were obtained from Yazd Agricultural Research Center, Iran. Hulls were dried and ground, and then a fraction that was sieved through a 10-mesh sieve and retained on a 40-mesh sieve was selected and freeze stored at −20°C until extraction. All chemicals were of analytical grade, obtained from Merck (Darmstadt, Germany) and used without any further purification.

Selection of Relevant Variables and Experimental Ranges

Before the development of the study through Response Surface Methodology (RSM), a first set of tests were performed to select the relevant factors (temperature, time and ratio) which are effective on phenolic extraction yield (dependent variable) and the experimental ranges for these independent variables.

In general, efficiency of the extraction of a compound is influenced by such multiple parameters as temperature, time and solvent polarity, and their effects may be either independent or interactive (Myers and Montgomery, 2002). According to the previously published papers on the extraction of phenolic compounds from different samples; in this study, effect of different solvents (with different polarities), (water, ethanol, acetone, methanol, ethyl acetate, 2-Propanol, methanol/water (70/30), ethanol/water (70/30), methanol/water/acetic acid (70/30/1) and ethanol/water/acetic acid (70/30/1)) was investigated on the total phenolic extraction from PGH. The results revealed that water is the most siutable solvent for the extraction of phenolic compounds. Therefore, water was selected as solvent for the next steps in the study. The size of the particles is another important variable that must be taken into consideration. With regards to initial experiments, the particle size of 10-40 mesh was selected as the most suitable in our study.

At a first step, the effect of liquid-to-solid ratio on the extraction was investigated by considering four ratios (10:1, 15:1, 20:1, 25:1; v/w). One g of milled hull was subjected to different ratios of water, during 30 minutes and at 25°C. Then, the effect of temperature on the Total Phenolic Content (TPC) was studied over the range of 25 to 85°C, using a liquid-to-solid ratio of 20:1 (20 ml of water 1 g^{-1} of milled hull), and a contact time of 30 minutes. Finally, the effect of time was studied to select the proper time range necessary for the extraction (ratio 20:1 v/w and 25°C).

Maceration

ME procedure was employed for the extraction of TPC from pistachio hull. Thus, according to the experimental design (for optimization), 1 g of milled hull was added to different volumes of water, then the mixture being kept at constant temperature. After a lapse of the needed suitable time for all treatments, 10 ml of solvent was being added to the initial volume, exactly before filtration and then the extract immediately filtered, and freeze stored in a freezer.

Ultrasound-Assisted Extraction (UAE)

An open rectangular ultrasonic bath (Elma Model TP690/H, 35 kHz, Germany) with internal dimensions of 50×14×10 cm was employed to carry out the extractions. The temperature was controlled and maintained at ±1°C by circulating external water from a thermostated controlled water bath. Other conditions were similar to those coming under Maceration section.

Microwave-Assisted Extraction (MAE)

The process of MAE was performed with the use of a household microwave (AEG Model EEH8223, Germany) at a frequency of 2,450 MHz and with some modifications for measuring and controlling of temperature (the microwave was equipped with a sensitive temperature sensing device and a digital controller). Other conditions and the next stages followed those in Maceration section.

Determination of Total Phenolics Content (TPC) in the Extracts

The TPC concentration was determined using Folin-Ciocalteu as a color reagent (Waterhouse, 2002). UV–Vis spectrophotometer (SINCO, Seoul, South Korea) was employed for a measurement of absorbance. Measurements were carried out in triplicate with the calculations being based on a standard curve based upon gallic acid (Sigma, St. Louis, MO). Total phenolic compounds were expressed as mg of gallic acid equivalents (GAE) per g of dry matter (DM).

Experimental Design

Optimization of extraction conditions of phenolics from PGH was carried out using RSM (Myers and Montgomery, 2002). A Central Composite Design (CCD) consisting of eighteen experimental runs was employed including four replicates at the center point. All the runs were carried out in duplicate. The design variables were the time (X1, min), extraction temperature (X2, °C) and liquid to solid ratio (X3) while the dependent variable being TPC (Table 1).

The response surface regression (RSREG) procedure of statistical analysis system

Table 1. Independent variables and their coded and actual values used for optimization.

Independent variable	Symbol	Coded level		
		-1	0	+1
Time (Min)	*X1*	13	25	37
Temperature (°C)	*X2*	33	45	57
Liquid/Solid ratio	*X3*	8	12.5	17

(SAS) and Statistica (Version 6) software were employed to analyze the experimental data. Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} b_{ji} X_i X_j$$

Where b_0, b_i, b_{ii}, and b_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and Xi, and Xj the independent variables. Statistica software was employed to generate response surfaces and contour plots while holding a variable constant in the second-order polynomial model.

Optimal conditions for the extraction of phenolic compounds from PGH depended on extraction time, extraction temperature, and liquid to solid ratio course, obtained using the predictive equations of RSM. TPC was determined following the extraction of phenolic compounds under optimal conditions. The experimental and predicted values were compared to determine the validity of the model.

RESULTS AND DISCUSSION

Liquid-to-Solid Ratio

The impact of the liquid-to-solid ratio on the extraction of phenolics from PGH was tested using four ratios (10:1, 15:1, 20:1, 25:1; v:w) over a 30 minutes extraction period with water, and at 25°C. The amount of TPC extracted per g of dried matter (DM) is presented in Figure 1A. Regression analysis demonstrated that quadratic (r= 0.99) and linear (r= 0.921) models better expressed the relationship between TPC and liquid-to-solid ratio than the other examined models. Also, the results of the one-way Analysis of Variance revealed that there were significant differences among the ratios studied. Duncan test indicated that there was no statistically significant difference between the ratios 20:1, and 25:1. According to the obtained results, it was demonstrated that whichever the ratio chosen above 20:1, the quantity of phenolic compounds extracted will remain the same. This allows for choosing any value above this limit, but one should avoid the use of an excessive quantity of solvent in the design of a process, because of economical aspects (higher solvent consumption and higher consumption of energy for extraction and concentration).

Effect of Temperature

The effect of temperature on the TPC extraction level was investigated over the range of 25 to 85°C. The level of TPC is presented in Figure 1B. Regression analysis demonstrated that quadratic (r= 0.945) and linear (r= 0.892) models were better acted than the other examined models for relationship between TPC and extraction temperature. According to the results, there was no significant difference ($P< 0.05$) observed between 65 and 85°C. Therefore, a fixed maximum temperature (axial value) of 65°C was adopted.

Effect of Time

The kinetics of polyphenol extraction was looked for to know the extraction rate and to allow for an appropriate choice of the experimental range to be included in the RSM for the variable of time. The main concern related to this question was to avoid a range of time leading to low variability levels in the yield of phenolics for a given temperature. On the basis of Figure 1C, the kinetics could be divided into two parts: a fast step, which takes around 20 minutes, and a slow step, for the rest of the studied period. Similarly, modelization for the extraction of antioxidants from *Melissa*

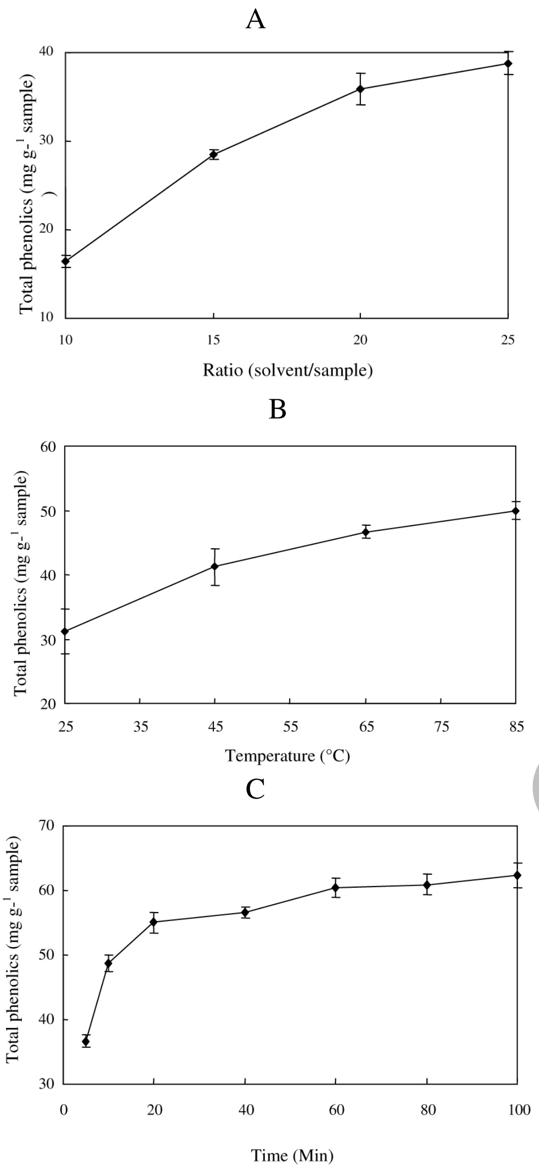

Figure 1. Effect of different extraction conditions on the extraction yield: (A) Liquid-to-solid ratio (30 minutes, water, and 25°C); (B) Temperature (30 minutes, water, and ratio 20:1 (v:w)), (C) Time (water, ratio 20:1 (v:w), and 25°C).

officinalis and *Inga edulis* leaves has successfully been performed in dividing the extraction phenomenon into two phases (Herodez *et al.,* 2003; Silva *et al.,* 2007). The regression analysis demonstrated that Sigmoid (r= 0.988) was more suitable than the other examined models for the relationships between TPC and extraction time. According to Figure 1C, following a lapse of 40 minutes yield of extraction was almost the same, also there was no significant difference (P< 0.05) observed between 40 and 60 minutes. Thus, the choice of a longer time can lead to no significant effect on this variable, as verified in the literature dealing with experimental design optimization (Pinelo *et al.,* 2005). According to these results, a maximum time (axial value) of 45 minutes was determined as fixed.

Fitting the Models

The three factors and lower, middle and upper design points for RSM in coded and actual/uncoded values are shown in Table 1. Multiple regression equations were generated relating the response variable to coded levels of the independent variables. Multiple regression coefficients were determined to predict quadratic polynomial models for TPC of pistachio hull extracts. Analysis of variance (ANOVA) showed that the selected quadratic models adequately represented the data obtained for TPC. The experimental design employed with the data for TPC of pistachio hull extracts examined is shown in Table 2. In addition, the corresponding coefficients of multiple determinations (R^2) for three methods of UAE, MAE, and ME are shown in Table 3. The models were adequate and explained most of the variability for the three methods.

A high proportion of variability was explained by the RSM models for TPC as indicated by R^2 (Table 3). The regression models were highly significant (P< 0.001 or P< 0.05) for the three methods with satisfactory coefficient of determination (R^2) namely: were 0.94, 0.95, and 0.96 for ME, UAE, and MAE, respectively.

An ANOVA of the regression parameters of the predicted response surface quadratic models for TPC of the three methods is shown in Table 4. The results indicated that both linear and quadratic parameters were highly significant (P< 0.001 or P< 0.05) for the three methods. However, interactions did

Table 2. Central composite design with the observed responses for ME, UAE, and MAE methods.

No	*X1* (t, Min)	*X2* (T,°C)	*X3* (Ratio)	Phenolics content (mg g^{-1} dw) ME [a]	UAE [b]	MAE [c]
1	13(-1)	33(-1)	8(-1)	26.9 ± 1.6b	33.3 ± 1.3a	27.2 ± 0.2b
2	13(-1)	33(-1)	17(+1)	39.5 ± 1.0b	43.9 ± 0.4a	39.2 ± 1.1b
3	13(-1)	57(+1)	8(-1)	31.6 ± 2.5a	32.4 ± 0.6a	33.7 ± 2.2a
4	13(-1)	57(+1)	17(+1)	43.7 ± 3.3b	53.5 ± 0.3a	47.8 ± 0.3ab
5	37(+1)	33(-1)	8(-1)	31.5 ± 1.3a	32.5 ± 0.1a	29.7 ± 1.3a
6	37(+1)	33(-1)	17(+1)	44.6 ± 2.5a	48.4 ± 2.1a	45.3 ± 1.0a
7	37(+1)	57(+1)	8(-1)	33.7 ± 1.8a	33.5 ± 2.6a	33.2 ± 2.2a
8	37(+1)	57(+1)	17(+1)	53.5 ± 0.2a	55.0 ± 2.6a	53.7 ± 0.5a
9	5(-1.68)	45(0)	12.5(0)	30.3 ± 0.2b	35.8 ± 1.1a	35.8 ± 2.8a
10	45(+1.68)	45(0)	12.5(0)	41.7 ± 0.5b	48.2 ± 0.4a	42.7 ± 1.1b
11	25(0)	25(-1.68)	12.5(0)	36.3 ± 2.0ab	40.8 ± 0.3a	33.5 ± 2.6b
12	25(0)	65(+1.68)	12.5(0)	47.5 ± 1.4ab	50.5 ± 0.4a	45.0 ± 0.4b
13	25(0)	45(0)	5(-1.68)	9.4 ± 0.9a	12.3 ± 1.6a	9.2 ± 2.2a
14	25(0)	45(0)	20(+1.68)	48.0 ± 0.2b	55.9 ± 1.2a	50.9 ± 0.2b
15	25(0)	45(0)	12.5(0)	38.5 ± 1.6b	46.1 ± 0.7a	45.1 ± 1.9b
16	25(0)	45(0)	12.5(0)	38.7 ± 0.5b	45.9 ± 0.4a	41.8 ± 0.9b
17	25(0)	45(0)	12.5(0)	44.6 ± 1.9b	45.7 ± 0.4a	41.1 ± 0.2b
18	25(0)	45(0)	12.5(0)	40.6 ± 1.6b	48.9 ± 1.8a	40.0 ± 1.5b

Values with different letters (a, b) in the same row are significantly different (P< 0.05, Duncan's Multiple Range Test).
[a] Maceration extraction; [b] ultrasound-assisted extraction, [c] Microwave-assisted extraction.

Table 3. Regression coefficients of predicted quadratic polynomial models for the response of TPC of ME, UAE, and MAE methods.

Coefficient	ME [a]	UAE [b]	MAE [c]
b_0	1723.04***	2174.29***	1761.06***
Linear			
b1	232.41***	173.34***	155.25***
b2	235.77***	226.77***	272.10***
b3	601.23***	793.06***	666.85***
Quadratic			
b11	-93.19*	-133.91**	-51.56ns
b22	68.48ns	-26.91ns	-44.68ns
b33	-139.8**	-192.57***	-127.80***
Crossproduct			
b12	46.267ns	-16.15ns	-11.44ns
b13	121.83*	70.14ns	125.30**
b23	100.47*	201.30***	118.54**
R^{2d}	0.94	0.95	0.96

*Significant at 5%; ** Significant at 1%, *** Significant at 0.1%, ns= Not significant.
[a] Maceration extraction; [b] ultrasound-assisted extraction; [c] Microwave-assisted extraction,
[d] Coefficient of multiple determination.

Table 4. Analysis of variance of the regression parameters of the predicted response surface quadratic models.

Regression	df	Sum of squares	Mean square	*F*-value
ME [a]				
Linear	3	12866999	4289000	125.9568***
Quadratic	3	925425.3	308475.1	9.059113***
Cross product	3	433254.6	144418.2	4.241188*
Total model	9	14225679	1580631	46.41903***
Lack of fit	5	271919.1	54383.82	1.861803 ns
Pure error	21	613416.2	29210.3	
UAE [b]				
Linear	3	19403909	6467970	176.8214***
Quadratic	3	1193739	397912.9	10.87815***
Cross product	3	731235.8	243745.3	6.663511**
Total model	9	21328884	2369876	64.7877***
Lack of fit	5	586403.8	117280.8	6.754086***
Pure error	21	364652.8	17364.42	
MAE [c]				
Linear	3	14826590	4942197	206.9644***
Quadratic	3	434497.7	144832.6	6.065155**
Cross product	3	478126	159375.3	6.674163**
Total model	9	15739214	1748802	73.23458***
Lack of fit	5	206402.3	41280.46	2.091595 ns
Pure error	21	414463.4	19736.35	

* Significant at 5%; ** Significant at 1%, *** Significant at 0.1%, ns= Not significant.
[a] Maceration extraction; [b] ultrasound-assisted extraction, [c] Microwave-assisted extraction.

not exhibit any significant effect in any of the cases. Thus, linear and quadratic effects of independent variables were the primary determining terms that may cause significant effects in the response while the interaction terms were insignificant in some cases. The positive coefficients for *X1, X2* and *X3* indicated linear effects that may increase the responses (Table 3). The quadratic effects of independent variables demonstrated both positive as well as negative effects.

Analysis of Response Surfaces

The regression models allowed the prediction of the effects of the three parameters on TPC of PGH for the three methods. The relationship between independent and dependent variables is illustrated in three dimensional representations of the response surfaces and two-dimensional contour plots generated by the models for TPC. Since time exhibited lower effect on TPC of the extracts from three methods in different circumstances, the response surface and contour plots were

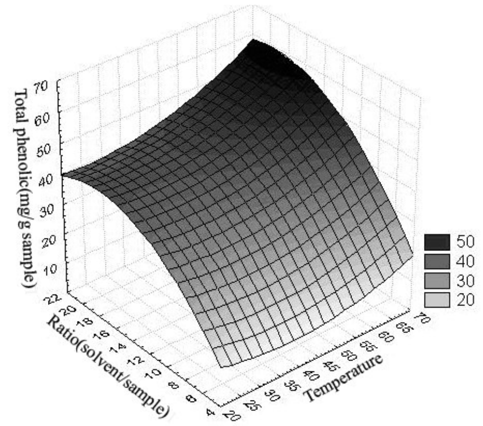

Figure 2. Response surface for the effects of extraction conditions on the extraction yield of phenolic compounds from PGH by ME. The value of the missing independent variable in each plot kept at the center point.

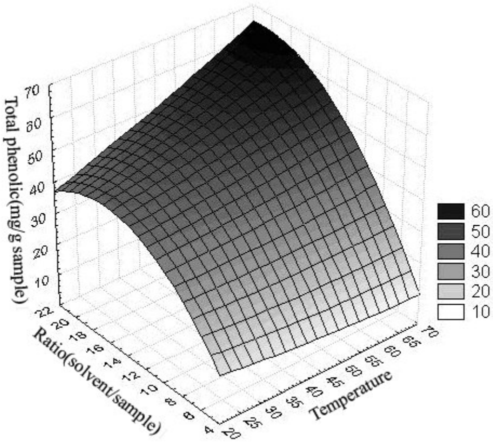

Figure 3. Response surface for the effects of extraction conditions on the extraction yield of phenolic compounds from PGH by UAE. The value of the missing independent variable in each plot kept at the center point.

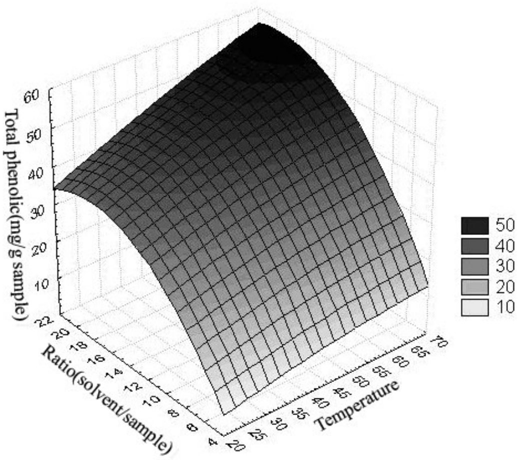

Figure 4. Response surface for the effects of extraction conditions on the extraction yield of phenolic compounds from PGH by MAE. The value of the missing independent variable in each plot kept at the center point.

generated as a function of liquid-to-solid ratio and temperature while keeping the time constant at 25 minutes. Figures 2, 3, and 4 depict response surface of the effects of the two variables, namely liquid-to-solid ratio and temperature on TPC of the extracts obtained through ME, UAE, and MAE, respectively. Figure 2 shows that ratio as well as temperature demonstrated quadratic effects on the response of ME method. In addition, Figures 3, and 4 show similar results as ME for UAE, and MAE methods.

Determination and Experimental Validation of the Optimal Conditions

In order to verify the predictive capacity of the model, an optimum condition was determined using the simple method and the maximum desirability for the phenolic contents extraction, (Table 5). The measured values lay within a 95% mean confidence interval of the predicted value for TPC, for the three methods (ME, UAE, and MAE). These results confirm the predictability of the model for the extraction of phenolics from PGH in the employed experimental conditions.

From a technological point of view, other conditions giving results close to those obtained for the optimum are frequently desirable. This is particularly demanding when there are some drawbacks related to the process, such as the use of a high quantity of solvent, or problems arising from the degradation of phenolics at undesirable high temperatures. In the Figures 2, 3, and 4 the darkest regions could be explored for the purpose.

Comparison between Extraction Methods

The results of the one-way Analysis of Variance showed that there existed a significant difference among ME, UAE, and MAE methods. Duncan test indicated that UAE was more efficient than the other methods studied, also there was a statistically significant difference between the UAE, and the other two methods. Jacques *et al*. (2007) demonstrated that in the extraction of *Ilex paraguariensis* leaves, UAE was more effective than ME. Also, other studies have verified that UAE could be used as a useful and efficient method for extraction of organic compounds (Fu *et al*., 2006; Hemwimol *et al*., 2006; Rodrigues and Pinto, 2006; Li *et al*., 2007; cho *et al*.,

Table 5. Comparison between predicted and experimental values (at optimum conditions) of the response variable in ME, UAE, and MAE methods.

Method	Independent variables			Predicted value	Observed value
	t (Min)	T (°C)	Ratio		
ME [a]	45	65	20	58.39	57.79 ± 0.15
UAE [b]	25	65	20	62.45	60.95 ± 0.69
MAE [c]	45	65	20	62.37	61.015 ± 1.43

[a] Maceration extraction; [b] ultrasound-assisted extraction, [c] Microwave-assisted extraction.

2006). Sound waves can create bubbles in a liquid and produce negative pressure. The bubbles form, grow and finally collapse. Close to a solid boundary, cavity collapse is asymmetric and produces high-speed jets of liquid. The liquid jets have strong impact on the solid surface (Luque-Garcia and Luque de Castro, 2003) therefore, it can increase the extraction rate. Gabaldo´n-Leyva *et al*. (2007) showed that ultrasound increased mass transfer of some compounds from red bell pepper because of increasing cell wall permeability. In addition, Table 2 shows the results of Duncan test among the three mentioned methods. According to the results presented in Table 2, in UAE method, ultrasonic waves in earlier times exhibited more efficacy than in later times. Therefore, ultrasonic waves show most effect in fast phase extraction while in the slow phase, the effect of ultrasonic waves is not conspicuous. These results are a verification of the results obtained by Rodrigues *et al*. (2008) and Balachandran *et al*. (2006).

CONCLUSIONS

The high correlation of the model exhibited that: second-order polynomial model could be employed to optimize the extraction of phenolic compounds from PGH through ME, UAE, and MAE methods for maximizing the yield of total phenolic compounds. Ratio, temperature, and time were found to be the most effective in extracting phenolic compounds in the three methods, respectively. Hence, the conditions for extraction of phenolics from PGH by ME, UAE, and MAE methods were 20(v/w), 65°C, 45 minutes; 20(v/w), 65°C, 25 minutes; and 20(v/w), 65°C, 45 minutes, respectively. Under optimized conditions the experimental values well agreed with the values predicted by models. In addition, UAE was more effective method than ME and MAE methods.

Abbreviations

PGH	Pistachio Green Hull
TPC	Total Phenolic Content
ME	Maceration Extraction
RSM	Response Surface Methodology
UAE	Ultrasound-Assisted Extraction
MAE	Microwave-Assisted Extraction

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بهینه سازی شرایط استخراج ترکیبات فنولیک از پوست سبز پسته(*Pistachia vera*) به روش سطح پاسخ

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چکیده

ترکیبات فنولیک، به‌ویژه آن‌هایی که منشا گیاهی دارند، به دلیل خصوصیات آنتی‌اکسیدانی‌شان بخش اساسی از رژیم غذایی انسان را تشکیل می‌دهند. در این تحقیق، روش‌های استخراج به کمک امواج فراصوتی، استخراج به کمک امواج مایکروویو و روش غرقابی به منظور استخراج ترکیبات فنولیک از پوست سبز پسته استفاده شد و به منظور بهینه سازی شرایط استخراج نیز از روش آماری سطح پاسخ کمک گرفته شد. برای تعیین اثرات متغییرهای مستقل از طرح مرکب مرکزی استفاده گردید که متغییرها شامل نسبت حلال به مواد جامد (۸-۲۰ برابر)، دما (۲۵- ۶۵ ^{o}C) و زمان (۵- ۴۵ دقیقه)بودند. نتایج نشان داد که در زمان استخراج مشابه، راندمان استخراج در روش استخراج به کمک امواج فراصوتی بیشتر از دو روش دیگر بود. ضریب همبستگی مدل‌ها برای روش‌های استخراج به کمک امواج فراصوتی، استخراج به کمک امواج مایکروویو و روش غرقابی به ترتیب ۰/۹۵، ۰/۹۶ و ۰/۹۴ بودند. شرایط بهینه برای استخراج ترکیبات فنولیک از پوست سبز پسته به وسیله روش‌های غرقابی، فراصوت و مایکروویو به ترتیب ۲۰(حجمی/وزنی)، ۶۵ درجه سانتی‌گراد، ۴۵ دقیقه؛ ۲۰(حجمی/وزنی)، ۶۵ درجه سانتی‌گراد، ۲۵ دقیقه و ۲۰(حجمی/وزنی)، ۶۵ درجه سانتی‌گراد، ۴۵ دقیقه بودند. در شرایط بهینه، مقادیر آزمایش شده با مقادیر پیش‌بینی شده توسط مدل‌ها مطابقت داشت.