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Research Paper / Araştırma Makalesi

Process Optimization for the Extraction of Phenolic Compounds from Pomegranate Peels: Response Surface Methodology-Desirability Function and Artificial Neural Network-Genetic Algorithm

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ABSTRACT

Valorization of agricultural wastes is ongoing topic in industry. Determining the best conditions by artificial neural networks based optimization techniques is the key step to extract valuable compounds efficiently and to obtain high quality extracts. In this study, the response surface methodology (RSM)-desirability function (DF) and artificial neural network (ANN)-genetic algorithm (GA) approaches were compared in modeling and optimization the extraction parameters (temperature, time and ethanol concentration (ratio of ethanol to water, % v/v)) of phenolic compounds in pomegranate peels. The ANN-GA approach providing higher coefficient of determination and lower root mean square deviation showed better predictive capability than the RSM. The optimum time (81.4 min) and ethanol concentration (15.7%) of RSM-DF approach shifted to the lower levels (78.8 min and 15.3%) with the ANN-GA approach while the optimum temperature (54.0°C) shifted to a higher level (59.3°C). The use of these values provided total phenolic content of >1000 mg GAE L^{-1} and the corresponding antioxidant activity was 11 mmol TE L^{-1}. As a result, increasing temperature up to a critical level decreased the extraction time and ethanol concentration, and it was determined that higher time-temperature combinations must be used for the complete water-based extraction of phenolic compounds from plant wastes in comparison to ethanol-water based extraction.

Keywords: Valorization pomegranate peel, Extraction, Response surface methodology, Artificial neural network, Genetic algorithm

Nar Kabuklarından Fenolik Bileşiklerin Ekstraksiyonunda Proses Optimizasyonu: Yanıt Yüzeyi Yöntemi-İstenebilirlik Fonksiyonu ve Yapay Sinir Ağı-Genetik Algoritma

ÖZ

Tarımsal atıkların değerlendirilmesi endüstride güncelliğini sürdüren bir konudur. Değerli bileşikleri verimli bir şekilde ekstrakte etmek ve yüksek kaliteli ekstraktlar elde etmek için en iyi koşulların yapay sinir ağları tabanlı optimizasyon teknikleri ile belirlenmesi önemli bir adımdır. Bu çalışmada, nar kabuklarındaki fenolik bileşiklerin ekstraksiyon parametrelerinin (sıcaklık, süre ve etanol konsantrasyonu (etanol/su oranı, % v/v)) modellenmesinde ve optimizasyonunda yanıt yüzeyi yöntemi (RSM)-istenebilirlik fonksiyonu (DF) ve yapay sinir ağı (YSA)-genetik algoritma (GA) yaklaşımları karşılaştırılmıştır. ANN-GA yaklaşımı daha yüksek determinasyon katsayısı ve daha düşük ortalama karekök sapması sağlayarak RSM'den daha iyi bir tahmin yeteneği göstermiştir. RSM-DF yaklaşımının optimum süresi (81.4 dakika) ve etanol konsantrasyonu (%15.7) ANN-GA yaklaşımı ile daha düşük seviyelere (78.8 dakika ve %15.3) kayarken optimum sıcaklık (54.0°C) ise daha yüksek bir seviyeye kaymıştır (59.3°C). Bu değerlerin kullanımı >1000 mg GAE L^{-1} toplam fenolik içerik ve 11 mmol TE L^{-1} antioksidan aktivite sağlamıştır. Sonuç olarak, sıcaklığın kritik bir seviyeye çıkarılması ekstraksiyon süresini ve etanol konsantrasyonunu

azaltmıştır ve bitki atıklarından fenolik bileşiklerin tamamen su bazlı ekstraksiyonunda etanol-su bazlı ekstraksiyonuna göre daha yüksek zaman-sıcaklık kombinasyonlarının kullanılması gerektiği belirlenmiştir.

Anahtar Kelimeler: Nar kabuklarının değerlendirilmesi, Ekstraksiyon, Yanıt yüzey yöntemi, Yapay sinir ağı, Genetik algoritma

INTRODUCTION

Extraction is known as a general method used to extract phenolic compounds from pomegranate peel as well as other natural plant sources. In the first step of the method, the solute and solvent are contacted to occur a mass transfer between the two phases up to equilibrium state. Initially, the solute is dissolved from the solid surface and then dispersed into the solvent by diffusion. In this stage, diffusion coefficient and dissolution rate are the main factors affecting extraction yield until reaching equilibrium concentration. In the second step, the solid residue is separated from the solvent which contain rich solute in the system [1]. Totally, pH, physicochemical structure of solute and solvent, particle size, surface area, temperature and contact time are known as the main parameters which were evaluated based on extraction yield and quality [2]. There are studies in the literature to investigate the effects of different combinations of these parameters on extracting phenolic compounds from pomegranate peel. Amyrgialaki et al. [3] determined that optimum extraction parameters were ethanol concentration of 40%, pH of 2 and time of extraction of 1 h at room temperature for the maximum total polyphenolic content and 97% of overall effect is accounted by ethanol concentration. Sood et al. [4] determined that extraction parameters were solid to solvent ratio of 1:30, temperature of 50°C and time of extraction of 45 min for the maximum total polyphenolic content using 60% ethanol concentration. These studies show that some of the extraction parameters have more impact on the process than others and that they interact with each other. By evaluating these interactions, it is possible to increase the extraction efficiency of temperature-sensitive compounds and the amount of use of green solvents or shorten the extraction times.

Phenolic compounds contain one or more hydroxyl groups (polar part) in their structure, which are bonded to an aromatic ring (apolar part) and they are usually not free in plant tissues, but in the form of esters or glycosides [5]. Differences in the molecular structure of the phenolic compounds cause changes in their polarity. Flavonoid aglycones and phenolic acids are well soluble in solvents such as diethyl ether, ethyl acetate [6] while the hydrolysable tannins are better dissolved in polar protic solvents such as hydroethanolic mixtures [7]. The rise in temperature increases the solubility and diffusion coefficient of the soluble compounds and the permeability of the cell wall [8] and higher efficiency is obtained by using longer period. However, high-temperature and long-time combinations cause to deteriorate structure of the extracted compounds [9]. Therefore, not only the selection of the solvent mixture considering its suitability to substrate material, its toxicity and residual limit value but also performing the process in optimum conditions are important steps to obtain maximum process efficiency and higher quality of final extract.

According to the foreseen effects of time-temperature combinations and solvent type on the extraction yield, carrying out the process under the best conditions is key step to obtain high quality extracts. Statistical and mathematical techniques such as response surface method (RSM) are used to evaluate interactions of independent variables with individual effects on the response, which is the main difference between the RSM and a single-factor experimental design [10]. The RSM was used intensively for the development, improvement, modeling and optimization of new or existing products [11]. Artificial neural network (ANN) which is an information processing concept inspired by the biological nervous systems [12], can be also used modeling linear and non-linear problems and by associating the single or multivariable inputs and a single or multivariable outputs [13]. Genetic algorithm (GA) is a non-mathematical optimization technique that obtain the solution of single and multi-objective optimization problems inspired by the biological evolution theory [14]. In the literature, studies that predict and model processes such as drying, extrusion, sterilization, membrane separation, extraction using ANNs [15-17], moreover studies which optimize processes using ANNs hybridized with GA are increasing [18-20]. These methods have been used together for both modeling and optimizing several extraction processes and it was shown that strong correlations with experimental results can be associated via developed models. Using the RSM and optimization with desirability function (DF) and ANNs hybridized with GA in extraction of phenolic compounds from pomegranate peels take attention by shortening the experimental study time, comprehensively explaining the contribution of extraction parameters to the process and making it easier to determine the optimum condition. To the best of our knowledge, there is no comparative study to investigate predictive capabilities of the RSM-DF and the ANN-GA on extraction of phenolic compounds from pomegranate peels.

In view of the above-mentioned facts, objectives of this study were: (i) evaluation of individual and interaction effects of temperature, time and solvent (ethanol) concentration on total phenolic content (TPC) and antioxidant activity (AA) of pomegranate peel extracts by Box Behnken method, (ii) modeling and optimizing the process with RSM-DF and ANN-GA approaches while reducing ethanol concentration and process temperature for maximum TPC and AA and finally (iii) comparing predictive capabilities of the approaches based on optimization results.

MATERIALS and METHODS

Materials

Fresh pomegranates were purchased from a local market in Edirne (Turkey). Peels were manually separated from the whole fruits. Fresh and ground peels were kept at - 25°C until the extraction procedure.

The main chemicals such as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol agent, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), gallic acid, sodium hydroxide, potassium persulfate, ethyl alcohol and sodium carbonate were purchased from Sigma (St. Louis, MO, USA). All the chemicals were of analytical grade.

Extraction Procedure

Outer peel (exocarp), spongy fleshy tissue (mesocarp) and white membrane layer (endocarp) parts of the pomegranate peels were separated from edible part (arils). These parts were chopped with electric chopper while fresh and stored at -25 degrees until extraction. Phenolic compounds from pomegranate peel were extracted in a shaking water bath (Memmert WNB22, Schawabach, Germany) with ±0.1°C adjustment precision. All samples were studied with the same shaking speed and 100 mL solvent volume. The extraction parameters were temperature (30, 55 and 80°C), time (10-50-90 min) and ethanol concentration (0-50-100%) and peel/solvent ratio was 1/30 (g mL^{-1}). The extracts were filtered by using a filter paper (Whatman Grade 5) and stored at - 25°C until the analysis.

Total Phenolic Content

Total phenolic content (TPC) was measured by the Folin Ciocalteu method [21]. The method based on the reduction of tungstate and/or molybdate in the Folin-Ciocalteu reagent by phenols. Blue product formation in alkaline medium by reduction is measured by a Shimadzu UV1800 spectrophotometer (Kyoto, Japan). 1 mL of sample, 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of 75 g L^{-1} sodium carbonate (Na_2CO_3) were mixed and the samples were incubated for 5 minutes at 50°C in a water bath (Memmert WNB22, Schawabach, Germany). Total phenolic content was calculated as gallic acid equivalent (mg GAE L^{-1}) by using absorbance values measured at 760 nm.

Antioxidant Activity

Antioxidant activity (AA) was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) assay [22] which is based on scavenging of ABTS•+ radical and measuring of decolorization at 734 nm. The ABTS•+ radical cation was prepared via reaction of 7 mM ABTS and 2.45 mM potassium persulfate (1/1, v/v) for 16 hours in dark. Firstly, the solution was diluted by using ethanol in order to provide absorbance of 0.70 (± 0.02) at 734 nm. After that, 30 µL sample was added to 3 mL diluted ABTS•+ radical. Finally, absorbance at 734 nm was recorded for 6 min. Total AA was expressed as TEAC (mmol TE L^{-1}) calculated by using percent absorbance reduction.

Experimental Design

The effects of temperature (T, °C), time (t, min) and ethanol concentration (%) on the TPC (mg GAE L^{-1}) and total AA (mmol TE L^{-1}) were investigated by using three level, three factor Box Behnken design. In order to determine the factors and their levels, previous studies in the literature were taken into consideration [3, 23]. Design-Expert® 11 (Stat-Ease Inc., Minneapolis, USA) statistical software was used to experimental design, modeling and optimization. The results of 15 runs (3 runs in the central point) were summarized in the Table 1. These results were used to both the RSM and the ANN studies.

Table 1. Box-Behnken experimental design and results of TPC and AA

Run	Independent Variables			Responses	
	(A) Temperature (°C)	(B) Time (min)	(C) Ethanol concentration (%)	TPC* (mg GAE L^{-1})	AA** (mmol TE L^{-1})
1	55(0)	50(0)	50(0)	945	10.41
2	55(0)	10(-1)	100(+1)	410	4.49
3	55(0)	50(0)	50(0)	915	10.10
4	30(-1)	90(+1)	50(0)	899	9.79
5	80(+1)	90(+1)	50(0)	722	7.34
6	80(+1)	50(0)	0(-1)	862	9.59
7	55(0)	90(+1)	100(+1)	519	5.14
8	80(+1)	50(0)	100(+1)	510	4.14
9	30(-1)	50(0)	100(+1)	341	3.75
10	30(-1)	10(-1)	50(0)	638	6.71
11	55(0)	10(-1)	0(-1)	808	8.33
12	30(-1)	50(0)	0(-1)	791	7.62
13	55(0)	50(0)	50(0)	928	10.27
14	80(+1)	10(-1)	50(0)	900	9.63
15	55(0)	90(+1)	0(-1)	996	10.95

*TPC: total phenolic content, **AA: antioxidant activity

Modeling and Optimization

Response Surface Methodology and Desirability Function Approach

Correlation between dependent variable and independent variables were modeled by the quadratic polynomial equation which was shown in Equation 1:

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

where Y is the dependent variable (TPC or AA), Xi and Xj are independent variables (temperature, time and ethanol concentration), β0 is the constant, βi is the linear, βii and βij are the interaction coefficient terms and ε is the error (residual) term.

Quadratic polynomial equation constitutes by least squares method. Least squares method is based on minimizing the sum of squares of residual terms. The least squares method assumes that the residual (ε) has zero mean and constant variance (σ^2) and is independently and normally distributed. The appropriate of the residuals to the assumptions in the evaluation of the model adequacy is tested by residual graphs [24].

ANOVA based on hypothesis testing was used to evaluation of models and model terms. In order to investigate models statistical data were analyzed with F-statistic. P-value that corresponds to the F-statistic was calculated and the significance of the model and model coefficient terms was examined at the 95% confidence level. In the 95% confidence level the significance of the model and model coefficients are less than 5% ($p<0.05$) indicating that the model and model coefficients are statistically significant. The DF approach was employed to maximize TPC and AA. The purpose of this approach is to transform all targeted responses into a collective response with the best fit and determine the independent variables that provide this response. In the case of more than one targeted response, the geometric mean of desirability functions is calculated [25].

Artificial Neural Network and Optimization by Genetic Algorithm

The ANN-GA studies were performed with MATLAB® 2017b (The MathWorks, Inc., Natick, USA). Neural network tool (nn-tool) was used to the creation of the network. Network type, training function, performance function and adaption learning function were feed forward back-prop, Levenberg-Marquardt backpropagation algorithm (trainlm), mean squared error (MSE) and gradient descent with momentum weight and bias learning function (learngdm) respectively. The 60%, 20% and 20% of data was used to for training, validation and test.

The network architecture consists of three layers which one layer was input layer with three neurons (temperature, time and ethanol concentration), one layer was hidden layer with seven neurons and one layer was output layer with two neurons (TPC and AA). The hyperbolic tangent sigmoid function (tansig) (Equation 2) was used for hidden layer and the linear function (purelin) (Equation 3) was used to output layer.

$$Tansig(n) = \frac{1}{1+e^{-2n}} - 1 \quad (2)$$

$$Purelin(n) = n \quad (3)$$

The relationship between output and input parameters and the role of weights and bias are defined by Equation 4.

$$\begin{bmatrix} TPC \\ AA \end{bmatrix} = purelin\left(LW\{2,1\} \times tansig\left(IW\{1,1\} \times \begin{bmatrix} Temperature \\ Time \\ Ethanol\ concentration \end{bmatrix} + b(1)\right) + b(2)\right) \quad (4)$$

where IW is the input weight matrix, LW is the layer weight matrix, the b is the bias.

GA optimization tool was used to maximize the output variables. The fitness function used for the GA optimization is given in Equation 5.

$$F = Y_{TPC} + Y_{AA} \quad (5)$$

where Y_{TPC} is the TPC variable and Y_{AA} is the AA variable from ANN model. The GA parameters for maximizing the optimization criteria TPC and AA were as follows: population types: double vector, creation function: feasible population, selection function: uniform, scaling function: rank, crossover function: scattered, mutation function: adaptive feasible. Since GA optimization is minimization based, fitness function was multiplied by -1 to turn the operation into a maximization problem.

Comparison of Predictive Capacities of the RSM-DF and the ANN-GA Approaches

The ability of RSM and ANN to predict the relationship between independent and dependent variables was evaluated using coefficient of determination (R^2), root mean square error (RMSE) and average absolute deviation (AAD%) as were given in Equation 6, 7 and 8, respectively.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_{exp} - Y_{pre})^2}{\sum_{i=1}^{n}(Y_{exp} - Y_{avg})^2} \quad (6)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Y_{exp} - Y_{pre})^2}{n}} \quad (7)$$

$$AAD\% = \frac{\sum_{i=1}^{n} \frac{|(Y_{exp} - Y_{pre})^2|}{Y_{exp}}}{n} \times 100 \quad (8)$$

Where Y_{exp} is the experimental value, Y_{pre} is the predicted value (by RSM and ANN); Y_{avg} is the average of the experimental values, n is the number of experiments.

RESULTS and DISCUSSION

Modeling with Response Surface Methodology

The goal of statistical modeling of effects of temperature, time and solvent mixture on extraction efficiency of phenolic compounds from pomegranate peels was determining the best conditions providing the highest total phenolic content (TPC) and antioxidant activity (AA). The linearity between TPC and AA reported by Wang et al. [23] was also observed in this study (Table 1). There was strong correlation ($R^2 = 0.9690$) between the TPC and the AA of the pomegranate peel extracts (PPE). The statistical analysis of the linear, two-factor interaction (2FI) and quadratic models generated with the least squares method were done (Table 2) and quadratic model was found to be the most appropriate model to explain the TPC and the AA of the PPE according to both p-value (p) < 0.0500 and lack of fit (LF) > 0.0500. Quadratic models had lower standard deviation (SD) and higher coefficient of determination (R^2), adjusted coefficient of determination (A-R^2) and predicted coefficient of determination (P-R^2) as compared with the linear and 2FI models. R^2 is a measure of the fit of the model. It expresses to what extent the dependent variables can be explained by the independent variables in the regression. When a new variable is added to the model the R^2 increases, whether this variable is statistically significant or insignificant. This will result in poor predictive power in evaluating new observations or answers, even though the model has a high R^2. Therefore, it is recommended to use the A-R^2 instead of the R^2. The A-R^2 does not increase when a new variable is added to the model and decreases when a insignificant variable is added. The high difference between R^2 and A-R^2 indicates that there are insignificant terms in the model. P-R^2 is a measure of the variation in new data explained by the model. As suggested the difference between the R^2 and the A- R^2 and the A- R^2 and the P-R^2 were less than 0.2 was a measure of the adequacy of the quadratic model [10]. In addition, the predicted residual error sum of squares (PRESS) values of both quadratic models were lower than the other models confirming desirability of the quadratic model to evaluate its prediction ability [10]. Furthermore, the coefficient of precision (adequate precision) measuring the signal/noise ratio of the models were found to be 16.80 and 16.32. This coefficient which compares the range of predicted maximum and minimum values with the average prediction error was greater than 4.00 is another indication that the models can be used to navigate the design space [26]. The coefficients of variation (CV: relative standard deviation) which expresses the percentage of the standard deviation relative to the mean were 5.95% and 6.41% and were below 10% as desired.

Table 2. Statistical data of the obtained models for TPC model and AA model

Model	p-value	LF	SD	R^2	A-R^2	P-R^2	PRESS
TPC_{Linear}	0.0129	0.0083	148.8800	0.6109	0.5048	0.3280	421100
TPC_{2FI}	0.5648	0.0071	154.7900	0.6941	0.4647	0.0491	595800
$TPC_{Quadratic}$	0.0012	0.0682	44.3800	0.9843	0.9560	0.7585	151300
AA_{Linear}	0.0289	0.0054	1.9100	0.5455	0.4215	0.2182	68.8500
AA_{2FI}	0.5541	0.0046	1.9800	0.6454	0.3795	-0.0826	95.3500
$AA_{Quadratic}$	0.0007	0.0561	0.5054	0.9855	0.9594	0.7755	19.7700

TPC: total phenolic content, AA: antioxidant activity, 2FI: two factor interaction, LF: lack of fit, SD: standard deviation, R^2: coefficient of determination, A-R^2: adjusted coefficient of determination, P-R^2: predicted coefficient of determination, PRESS: the predicted residual error sum of squares

For the analysis of model appropriate, the residual graphs of the TPC model and the AA model were shown in the Figure 1. One of the assumptions of the regression model is that the residuals have a normal distribution. The residuals that conform to normal distribution should be distributed near the normal line. As shown in the Figure 1a and 1g, the residuals showed a small scattering and showed a very close distribution to the theoretical normal distribution line. Constant variance and zero mean assumption were investigated in the Figure 1b and 1c for the TPC model and Figure 1h and 1i for the AA model, as shown in residuals were randomly distributed around the $y = 0$ and did not exceed 95% confidence level boundaries in the residuals against predicted and runs graphs.

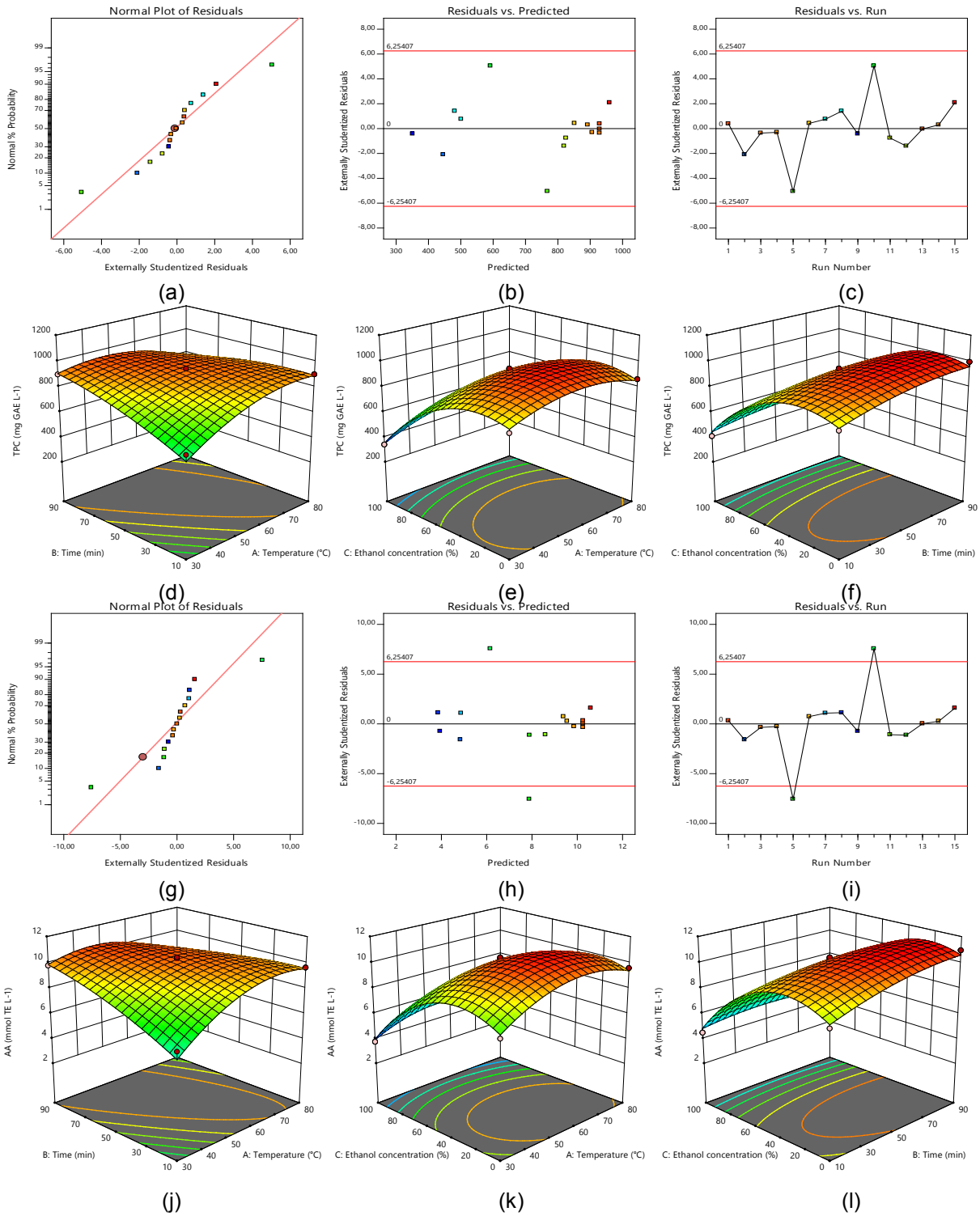

Figure 1. Residuals plots for **a-c** TPC, **g-i** AA, **a** and **g** normal plots of residuals, **b** and **h** residuals vs. predicted, **c** and **i** residuals vs. run, Response surface graphs for **d-f** TPC, **j-l** AA. **d** and **j** effect of temperature and time at constant ethanol concentration, **e** and **k** effect of temperature and ethanol concentration at constant time, **f** and **l** effect of time and ethanol concentration at constant temperature

ANOVA results for evaluating statistical significance of the model were reported in Table 3. The TPC model and the AA model was analyzed at 95% confidence level. The F-values of models were higher than F-critical values and the p-values of the models were determined less than 0.0500 (p=0.0006 and p=0.0005). Pure error was calculated by using 3 replicates at the center point. In lack of fit test, pure error can be used to test the significance of the residual variance sourced by the factors and their interactions that are currently in the model [27]. For lack of fit test, mean square of lack of fit and mean square of pure error calculated by dividing the sum of squares by the degrees of freedoms. F- values of lack of fit calculated by dividing mean square of lack

of fit by mean square of pure error. The p-values for the lack of fit of the models were 0.0682 and 0.0561. In hypothesis testing, the lack of fit value was insignificant (p>0.0500) means that the responses can be explained by regression equation. These findings showing the TPC model and the AA model were statistically significant. The TPC and the AA models with actual values were shown in Equation 9 and 10. As the p-value of model term decreases, the importance of the term on the model increases. Accordingly, we observed that linear effects of temperature, time and ethanol concentration, quadratic effects of temperature and ethanol concentration and interaction effect of the temperature and time were significant in TPC model and linear effects of time and ethanol concentration, quadratic effects of temperature and ethanol concentration and interaction effect of the temperature and time were significant in AA model.

$$TPC\ (mg\ GA\ L^{-1}) = -27.96792 + 23.45383\mathrm{A} + 10.29042\mathrm{B} + 3.41992\mathrm{C} - 0.109750\mathrm{AB} + 0.019600\mathrm{AC} - 0.009875\mathrm{BC} - 0.157467A^2 - 0.025729\mathrm{B}^2 - 0.081967\mathrm{C}^2 \quad (9)$$

$$AA\ (mmol\ Trolox\ L^{-1}) = -4.13965 + 0.347435\mathrm{A} + 0.128213\mathrm{B} + 0.084768\mathrm{C} - 0.001343\mathrm{AB} - 0.000316\mathrm{AC} - 0.000246\mathrm{BC} - 0.002276\mathrm{A}^2 - 0.000294\mathrm{B}^2 - 0.001025\mathrm{C}^2 \quad (10)$$

where A, B and C is temperature, time and ethanol concentration, respectively.

Effect of Independent Variables on the TPC and the AA of Extracts

Three-dimensional (3-D) response surface graphs, which were formed by plotting the response variable against two independent variables while other independent variable was kept constant at a medium level, were given in the Figure 1. In these graphs, the response is shown gradually from red to green, from high to low, and with a contour graph at the base. At constant ethanol concentration (50%), increase in temperature and time increased the TPC and the AA up to a certain level (Figure 1d and 1j). After the maximum point, further increase in both temperature and time had a negative effect on the TPC and the AA, which can be attributed to the structural deterioration of phenolic compounds in long-term extraction processes at high temperatures [28]. Although it was mentioned that increase in temperature increased the TPC while extraction time was not significant before by Demir et al. [29], both the linear effect of temperature and time were found to be significant in this study. However, only the linear effect of temperature was not significant in the AA model (Table 3). Even Fourati et al. [30] stated that a longer extraction time presents a negative effect on the TPC. However, beside the linear effect of contact time was significant term of the quadratic TPC model, temperature-time interaction was also important to improve extraction of phenolic compounds from pomegranate peels. It was also determined that the similar TPC and AA values were obtained in high temperature-low time and low temperature-high time combinations, that means the extraction time decreases with increase of the extraction temperature. For example, TPC of 900 mg GAE L^{-1} was achieved in 88 min at 30°C while it was achieved in 11 min at 75°C. Consequently, increasing the extraction temperature up to a level having no deteriorative effects will cause to save time.

The effects of temperature and ethanol concentration in the medium level of time (50 min) are given in the Figure 1e and 1k. As in the results of ANOVA, the effect of ethanol concentration on both the TPC and the AA was higher than the effect of temperature. The effect of ethanol concentration on extraction efficiency was also reported by previous studies [29, 30]. The available range of ethanol concentration was determined 10-30% in the similar studies. In this study, at medium level of time, the maximum TPC (985 mg GAE L^{-1}) was obtained at 25% ethanol concentration and 59°C temperature while the maximum AA (10.86 mmol TE L^{-1}) was obtained at 26% ethanol concentration and 60°C temperature. The least TPC and AA values were obtained when 100% ethanol concentration was used. Tsakona et al. [31] also reported that the polarity of the solvent used in the extraction process had a significant effect on the TPC of the extracts and that the polar phenolic compounds were better dissolved in ethanolic mixtures containing ethanol in the 25-75% range compared to pure ethanol. The increase in temperature positively affected the response when the contact time kept constant. The positive effect of temperature on extraction efficiency was probably due to the increase in dissolution and diffusion rates of solutes [32].

The effects of time and ethanol concentration at constant temperature (55°C) are shown in the Figure 1f and Figure 1l. According to point prediction, the TPC and the AA reached 1003 mg GAE L^{-1} and 11.1 mmol TE L^{-1}, respectively, at using 22% ethanol concentration while reached 961 mg GAE L^{-1} TPC and 10.6 mmol TE L^{-1} using 0% ethanol (pure water) and reached 512 mg GAE L^{-1} and 5.1 mmol TE L^{-1} using 100% ethanol at the same temperature and time (55°C and 80 min). It was stated before by Wang et al. [23] that using an appropriate ethanol concentration in the solvent instead of pure ethanol or pure water approximately doubled TPC and AA of extracts. The TPC of 883 mg GAE L^{-1} and the AA of 9.5 mmol TE L^{-1} were obtained at 10 min when 22% ethanol was used in the solvent while the same values were obtained in 27.7 min when pure water was used at 55°C. This result showed that using a certain level of ethanol in the solvent shortened the extraction time.

Table 3. Results of statistical analysis for TPC model and AA model

Source	Total Phenolic Content (TPC)					Antioxidant Activity (AA)				
	Sum of squares	Df	Mean square	F-value	p-value	Sum of squares	Df	Mean square	F-value	p-value
Model	6.168E+05	9	68530.35	34.80	0.0006*	86.80	9	9.64	37.76	0.0005*
A	13203.12	1	13203.12	6.70	0.0489*	1.00	1	1.00	3.92	0.1046
B	18050.00	1	18050.00	9.17	0.0292*	2.06	1	2.06	8.07	0.0362*
C	3.515E+05	1	3.515E+05	178.51	< 0.0001*	44.98	1	44.98	176.11	< 0.0001*
AB	48180.25	1	48180.25	24.47	0.0043*	7.21	1	7.21	28.22	0.0032*
AC	2401.00	1	2401.00	1.22	0.3198	0.6241	1	0.6241	2.44	0.1788
BC	1560.25	1	1560.25	0.79	0.4142	0.9702	1	0.9702	3.80	0.1088
A^2	35763.10	1	35763.10	18.16	0.0080*	7.47	1	7.47	29.25	0.0029*
B^2	6257.33	1	6257.33	3.18	0.1348	0.8156	1	0.8156	3.19	0.1340
C^2	1.550E+05	1	1.550E+05	78.73	0.0003*	24.25	1	24.25	94.92	0.0002*
Residual	9846.42	5	1969.28			1.28	5	0.2554		
Lack of Fit	9393.75	3	3131.25	13.83	0.0682	1.23	3	0.4096	17.00	0.0561
Pure Error	452.67	2	226.33			0.0482	2	0.0241		
Cor Total	6.266E+05	14				88.08	14			

A: temperature (°C), B: time (min), C: ethanol concentration (%) Df: degrees of freedom, *significant ($p<0.05$)

Optimization with DF Approach

The extraction process was optimized for achieving the maximum TPC and AA. The lower and upper limits of the operating variables were kept at – 1 and + 1 levels in the experimental design. The lower and upper limits of the responses were equal to the lowest and highest values obtained in the experiments (341-996 mg GAE L^{-1} for TPC and 3.75-10.95 mmol TE L^{-1} for AA). While the importance level of all operating variables (temperature, time and ethanol concentration) was determined as 3, with the same importance, the importance level of the responses (TPC and AA) was determined as 5, with the same importance level and higher than the operating variables. Optimum conditions for the extracting phenolic compounds from pomegranate peels by solid-liquid method were temperature of 54.0°C, contact time of 81.4 min and ethanol concentration of 15.7% providing 1002 mg GAE L^{-1} TPC and 11.1 mmol TE L^{-1} AA.

Modeling with ANN

The ANN was also used to determine the relationship between the independent variables (temperature, time and ethanol concentration) and the dependent variables (TPC and AA). The experimental data of RSM study was used in the ANN study as input layer and output layer. Optimum neuron number of hidden layer was determined to be seven by trial and error method using the experimental data [33]. The testing of the model was carried out with the scatter plot between target and output data for training, validation, testing, and all data with the Pearson's correlation coefficient (R) were shown in the Figure 2a, 2b, 2c and 2d. The R value was 0.99954, 0.99861, 1.00000, 0.99917 for training, validation, testing, and all data, respectively. The approaching of the R to 1 indicates a strength and positive relationship between target and output.

$$IW\{1,1\} = \begin{bmatrix} 1.84448 & 2.50100 & 1.33054 \\ -0.55856 & 0.90620 & -1.69485 \\ -2.41696 & 0.97652 & -1.74415 \\ 0.25289 & 2.29764 & -1.80169 \\ -0.05689 & -1.79711 & -1.08251 \\ 0.25015 & 2.61598 & 3.54671 \\ 0.26070 & 1.46703 & 1.50553 \end{bmatrix} \quad (11)$$

$$LW\{2,1\} \begin{bmatrix} -0.10740 & -0.98973 & -0.11166 & 1.28154 & -0.97385 & -1.46273 & 0.04531 \\ -0.54899 & -0.4\mathrm{E}-05 & -0.28944 & 0.81503 & -1.16563 & -1.50773 & -0.26878 \end{bmatrix} \quad (12)$$

$$b(1) = \begin{bmatrix} -4.30325 \\ 2.74390 \\ -0.61207 \\ 3.26872 \\ 0.85750 \\ -2.47740 \\ -3.40283 \end{bmatrix} \quad (13)$$

$$b(2) = \begin{bmatrix} -0.39242 \\ -1.652989 \end{bmatrix} \quad (14)$$

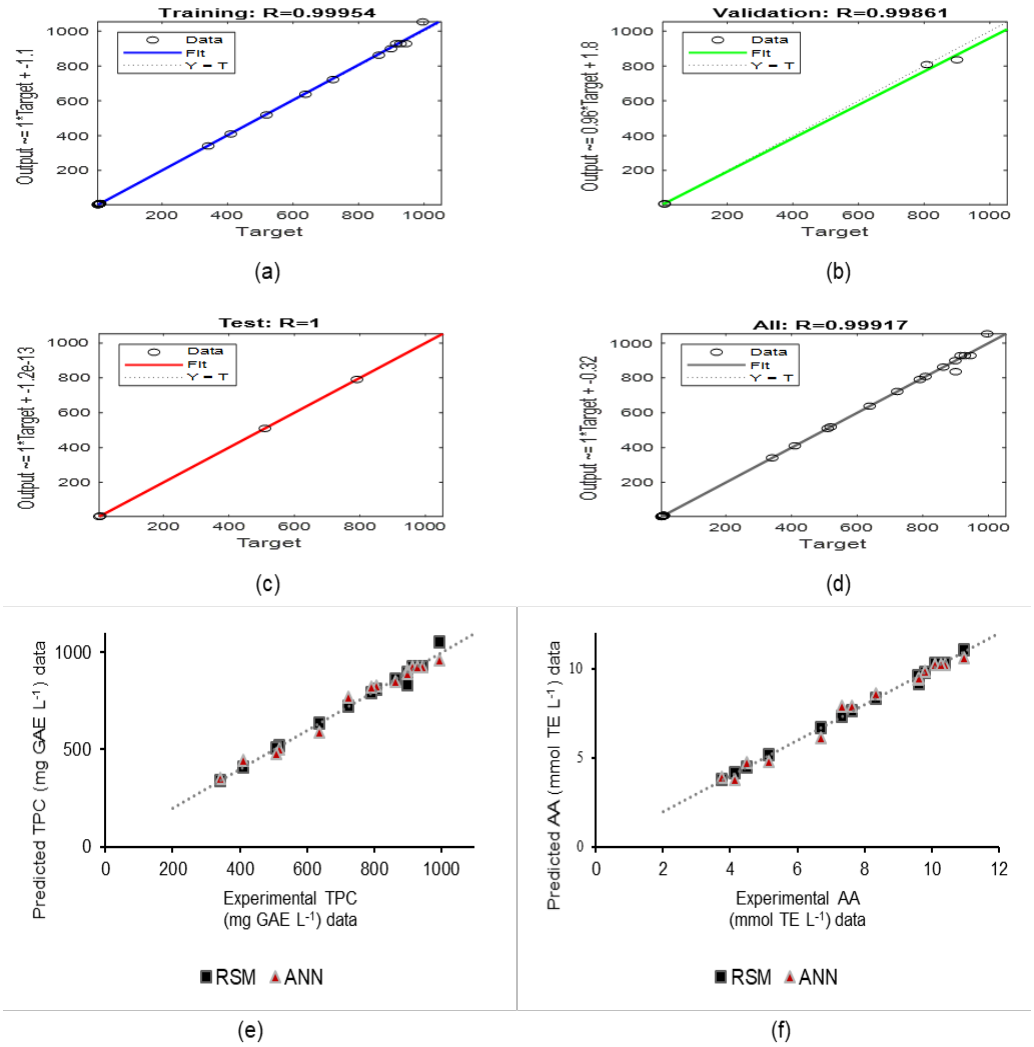

Figure 2. Scatter plot between experimental target and ANN output for **a** training, **b** validation, **c** testing, and **d** all data, scatter plot between experimental data and RSM and ANN predicted data for **e** TPC, **f** AA

The optimum weights and bias values used to predict the output data by minimizing the error between target and output data were given in Equation 11-14. The IW was the 7x3 weight matrix connecting the neurons of input layer to the hidden layer. The IW had 7 rows and 3 columns because the number of neurons was 7 and the number of independent variables was 3. The LW was the 2x7 weight matrix connecting the neurons of hidden layer to the output layer. The LW had 2 rows and 7 columns because the number of neurons was 7 and the number of dependent variables was 2. b(1) was the 7x1 bias column vector of the neurons of the hidden layer and b(2) was the 2x1 bias column vector of the neurons of the output layer.

Optimization with GA

The optimization was performed with three variables which lower bounds (30, 10 ,0), upper bounds (80, 90, 100). The TPC and the AA maximized after 51 iterations with GA. According to the optimization results performed with GA, the optimum conditions were temperature of 59.3°C, contact time of 78.8 min and ethanol concentration of 15.3% providing TPC of 1012 mg GAE L^{-1} and AA of 10.7 mmol TE L^{-1}.

Comparison of RSM and ANN Methods

The predictive capabilities of the techniques were compared with the values of coefficient of determination (R^2), root mean square (RMSE) and average absolute deviation (AAD%) values given in the Table 4. In both the methods, values of R^2 very closed to 1, values of RMSE and AAD% were low as required. The experimental data versus predicted data graphs for the RSM and the ANN were also shown in the Figure 2e and 2f. Accordingly, the values predicted to the model showed a linear distribution with the actual data so the ability to predict the fitted model was high. Depend on these results, the predicted data with the RSM and the ANN correspond to the experimental data, both the methods could be used for estimation. As in similar studies summarized in the Table 4, R^2 of ANN for both the TPC and the AA were closer to 1 than RSM, RMSE and AAD% of ANN for both responses were lower than RSM indicates that ANN has improved predictive capability [33-35].

Table 4. Statistical evaluation for RSM's and ANN's predictive capability

Responses	RSM Model			ANN model			Reference
	R^2	RMSE	AAD%	R^2	RMSE	AAD%	
TPC	0.9843	25.62	3.32	0.9878	22.85	1.07	This study
AA	0.9855	0.30	3.88	0.9965	0.14	0.62	
TPC	0.9148	0.35	6.37	0.9930	0.10	2.17	[33]
AA	0.8806	0.10	7.00	0.9887	0.03	2.15	
TPC	0.9443	23.45	8.29	0.9833	12.93	3.66	[34]
AA	0.9742	3.07	5.64	0.9803	2.69	3.38	
TPC	0.9791	17.10	1.86	0.9851	14.82	1.50	[35]
AA	0.9666	1.28	0.15	0.9764	1.20	0.14	

RSM: response surface methodology, ANN: artificial neural network, TPC: total phenolic content, AA: antioxidant activity, R^2: coefficient of determination, RMSE: root mean square error, AAD%: average absolute deviation

CONCLUSION

Solid-liquid extraction method has a good potential to recover phenolic compounds from pomegranate peels. The optimum levels of independent variables of the process were successfully predicted by the RSM-DF and the ANN-GA approaches. It was showed that the ANN can generate better predictions by processing experimental data although both the methods have similar functions. Additionally, the individual and interaction effects of the factors on the process efficiency were well described by the RSM. Based on these results, it is recommended to use various approaches in optimization studies and evaluate them according to the characteristics of the study. The experimental data were represented by a quadratic-second order model. Using ethanol (in the range of 15-25%) in the solvent mixture provided the highest extraction yield. On the other hand, higher time-temperature combinations were needed to obtain a sustainable water base extraction method of high value phenolic compounds compared to ethanol-water base extraction. Further studies are still needed to evaluate predictive capability of the ANN-GA and increase the diversity of studies on comparing the results of the ANN-GA method with the results of different modeling and optimization method in optimization of alternative green extraction processes of bioactive substances from several plant-based wastes.

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