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Microwave-Assisted Extraction of *Prunus cerasus* L. Peels: Citric Acid-Based Deep Eutectic Solvents

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Abstract: In the present study, waste by-products of one of the most popular fruit juices in the World has been valorized for its high-added value ingredients. Peels of sour cherry (Prunus cerasus L.) have been extracted by deep eutectic solvent (DES)-based microwave-assisted extraction method (MAE). DES system contained citric acid as hydrogen bond acceptor (HBA) and ethylene glycol as hydrogen bond donor (HBD) (1/4, molar ratio). In order to optimize the MAE system Central Composite Design (CCD) of Response Surface Method (RSM) has been used. The measured variables were the yields of total phenolic content (TPC), total anthocyanins (TA), and cyanidin-3-glucoside. Optimum conditions were determined as 0.1 g of peel and a 50 % (v/v) water contribution to the DES for the maximum recovery of TPC (16.85 mg-GAE/g-FP), TA (3.39 mg-cyn-3-glu/g-FP) and cyanidin-3-glucoside (mg/g-FP) in the MAE of sour cherry peels. The relationship between the responses was also established.

Keywords: Green chemistry; deep eutectic solvents; waste by-products; optimization; anthocyanins.

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INTRODUCTION

The valorization of waste by-products from agri-food products has been a popular issue recently. The most consumed fruits, such as citrus peels (1–3), banana peels (4), apple peels (5), mango peels (6), apricot and peach peels (7), pomegranate peels (8), and sour cherry peels (9), were investigated in terms of extraction and their bioactive properties. Since Turkey is one of the primary countries in the world producing sour cherries (10), its peel has been selected to observe its polyphenol ingredients in the current study. Its medicinally therapeutic properties, such as antimicrobial (11), antioxidant, anti-tumor (12), anti-cancer, and anti-inflammatory (13) effects, have also been proven by various studies. Therefore, recovery of the related fine chemicals from biomass has been a very valuable issue nowadays. In order to propose a green process for obtaining the bioactive ingredients from sour cherry peels, microwave-assisted extraction (MAE) method has been adopted in the present study. The advantages of the MAE over conventional methods are demonstrated in Figure 1 (14). On the other hand, a deep eutectic solvent (DES) including citric acid (hydrogen bond acceptor) and ethylene glycol (hydrogen bond donor) was integrated into the MAE to be able to propose a more environmentally friendly procedure.

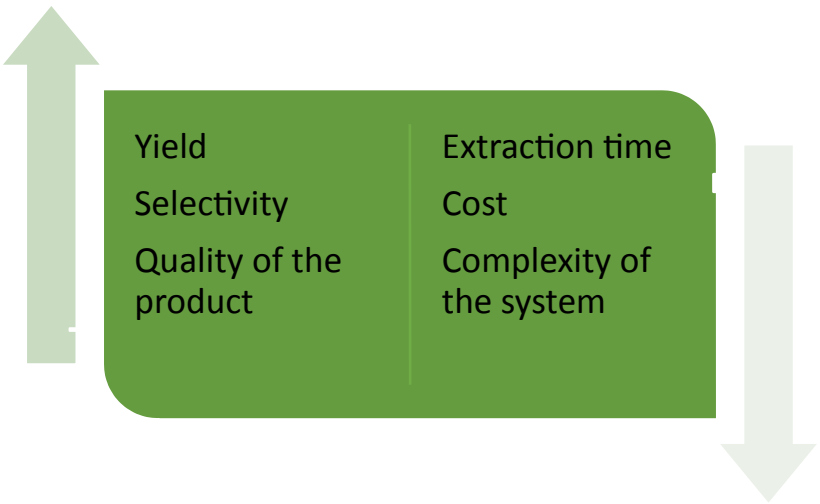


Figure 1: Advantages of MAE.

In order to optimize the process conditions to achieve the maximum yields of extraction, central composite design (CCD) of response surface method (RSM) has been exploited. The yields of the system have been evaluated according to the total phenolic content (TPC), total anthocyanin (TA), and cyanidin-3-glucoside level. Cyanidin-3-glucoside was particularly chosen due to the fact that it is one of the primary phenolic compounds in the sour cherry peel extract (9,15). The independent parameters were selected as water amount in the DES and mass of raw material, where extraction time and microwave power were determined depending on the preliminary experiments. The relationship between the dependent variables was also presented in terms of correlation coefficient.

MATERIAL AND METHODS

Materials

Citric acid (≥99.5%), ethylene glycol (≥99.5%), methanol (≥99.9%), ethanol (≥99.9%), and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, sodium carbonate (≥99.0%), gallic acid (≥98.0%), formic acid (≥98.0%), acetonitrile (≥99.9%), and cyanidin-3-glucoside were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Regarding plant material (sour cherry), the samples were obtained from DIMES Food located in Turkey. The peels were kept at -20 °C after being separated from the fruits. Fresh samples were used in the study.

DES Preparation

A heating and mixing method was adopted to prepare the selected system (¼ molar ratio, citric acid/ethylene glycol) depending on the previous study (16). Citric acid was used as hydrogen bond acceptor (HBA), whilst ethylene glycol was the hydrogen bond donor (HBD) in DES mixture.

MAE Procedure

MAE of sour cherry peels was performed by a laboratory scale microwave system (NEOS-GR, Milestone Srl, Italy). Table 1 shows the selected parameters and their levels used in this study. The effects of water addition to the DES and the amount of raw material were investigated. The power of the microwave was kept stable at 500 W for 3 min of extraction time with 50 mL of solvent, depending on the preliminary experiments.

Table 1: Process parameters of MAE for the extraction of bioactive substances from sour cherry peels.

Parameter	Symbol	Code with level		
		−1	**0**	**1**
Mass (g)	A	0.1	0.3	0.5
Water ratio (%, v/v)	B	20	35	50

Chromatographic and Spectrophotometric Analysis

The cyanidin-3-glucoside content of extracts was determined by Agilent 1260 chromatographic equipment (Agilent, Waldbronn, Germany), while TPC and TA of the sour cherry extracts were analyzed spectrophotometrically using a UV spectrophotometer (PG Instruments, T60/Leicestershire, England). The detailed information on high-performance liquid chromatography (HPLC) method for the quantification of cyanidin-3-glucoside was reported in the earlier paper (15).

Spectrophotometric measurements of TPC were performed at 765 nm. Extracted samples were

diluted with water first. Then, Folin-Ciocalteu reagent and sodium carbonate solutions were added and left for incubation for 30 min (17). The output was given in gallic acid equivalents per gram of fresh plant sample (mg-GAE/g-FP). On the other hand, spectrophotometric measurements of TA were carried out at 520 and 700 nm (9). The output was given in cyanidin-3-glucoside equivalents per gram of fresh plant sample (mg-cyn-3-glu/ g-FP).

RSM

The extraction process was modeled using the response surface approach. By applying a mathematical and statistical technique, RSM was provided with an association between the variables. Table 1 gives the selected independent parameters with their levels. In this research, RSM was exploited to optimize the amount of TPC, TA, and cyanidin-3-glucoside by using Design expert program (12th edition). Central composite design was selected as subtype of RSM. ANOVA (Analysis of Variance) test was employed to analyze the means of three replicates on Prism 9 software (GraphPad, San Diego, CA). Tukey's test was used to measure the significance between means through.

RESULTS AND DISCUSSION

Table 2 presents the experimental design and the findings depending on the conditions for the extraction of biomolecules from sour cherry peels by MAE process. Central composite design (CCD) was selected as subtype of RSM. 13 experimental runs were designed by the CCD for the MAE system with 3 factors and 2 levels.

Table 2: Experimental results formed by CCD*.

	A: Mass (g)	B: Water ratio (%, v/v)	TPC (mg-GAE/g-FP)	TA (mg-cyn-3-glu/g-FP)	Cyanidin-3-glucoside (mg/g-FP)
1	0.5	35	9.68±0.001^{a}	2.08±0.000^{a}	2.10±0.002^{a}
2	0.3	35	9.28±0.002^{b}	2.12±0.001^{b}	1.00±0.002^{b}
3	0.1	50	16.62±0.000^{c}	3.31±0.001^{c}	5.49±0.001^{c}
4	0.3	35	9.28±0.003^{d}	2.20±0.002^{d}	1.04±0.003^{d}
5	0.3	35	9.56±0.001^{e}	2.24±0.003^{e}	1.36±0.001^{e}
6	0.3	50	9.67±0.002^{f}	2.47±0.004^{f}	1.67±0.000^{f}
7	0.1	35	17.70±0.001^{g}	3.22±0.002^{g}	4.35±0.001^{g}
8	0.5	50	8.45±0.000^{h}	2.21±0.003^{h}	2.58±0.002^{h}
9	0.1	20	18.14±0.004^{i}	2.69±0.002^{i}	3.91±0.001^{i}
10	0.3	35	7.96±0.001^{j}	2.10±0.003^{j}	1.15±0.003^{j}
11	0.5	20	7.14±0.000^{k}	1.85±0.001^{k}	2.65±0.001^{k}
12	0.3	20	7.15±0.001^{l}	1.57±0.000^{l}	0.72±0.000^{l}
13	0.3	35	9.89±0.001^{m}	2.35±0.001^{m}	1.03±0.001^{m}

* Data are given as the mean (3 replicates) ± standard deviation

**The values with different superscript letters in a column are significantly different ($p<0.05$).

Modeling Study

Quadratic models derived for MAE system for the extraction of phytochemicals from sour cherry peels are given below (Eqs. 1-3):

$$\mathbf{Y_{TPC}=;} 9.24 - 4.53A + 0.3841B + 0.7075AB + 4.34A^2 - 0.9359B^2 \quad \text{(Eq. 1)}$$

$$\mathbf{Y_{TA}=;} 2.20 - 0.5126A + 0.3144B - 0.0649AB + 0.4717A^2 - 0.1603B^2 \quad \text{(Eq. 2)}$$

$$\mathbf{Y_{Cyanidin\text{-}3\text{-}glucoside}=;} 1.07 - 1.07A + 0.4120B - 0.4120AB + 2.28A^2 + 0.2488B^2 \quad \text{(Eq. 3)}$$

ANOVA (analysis of variance) test results for 3 responses (TPC, TA, and cyanidin-3-glucoside) are given in Table 3. All of the quadratic models generated for TPC, TA, and cyanidin-3-glucoside are statistically significant ($P < 0.0001$) at 95% of confidence interval. The coefficients of determination of second-order equations are extremely strong ($R^2 > 0.97$), which means that 97.12%, 96.28%, and 99.28% of the variabilities in the Y (response) values can be described by the quadratic models. Moreover, R^2s are in reasonable agreement with the adjusted R^2s, supporting the

significance of the derived models. Moreover, a non-significant lack of fit also backs the models up ($P > 0.05$). Furthermore, the coefficient of variation (<10%) demonstrates the reliability of the experimental output.

Optimization Study

The ANOVA table (Table 3) also provides us with an identification of the effects of process parameters on the responses. P-values of less than 0.05 demonstrate the importance of the terms. Generally, the amount of the raw material has been found to be the most effective at $P<0.0001$ for the three responses. On the other hand, the second order was much more effective for the third response (cyanidin-3-glucoside) considering its highest F-value.

Design-Expert software also has the opportunity to provide us with 3D (three dimensional) images in order to comprehend these process effects visually. Figures 2a, 2b, and 2c show the effects of process parameters (amount of peels and water content in the DES) on the MAE system for TPC, TA, and cyanidin-3-glucoside yields, respectively. In general, increasing the mass resulted in lower yields. This might be expectable due to the mass transfer phenomenon, where the amount of solvent in contact with the solid material decreases. Regarding water addition into the DES, water's amount in the solvent enhanced the extraction in terms of all dependent variables. Water has an important role in deep eutectic mixtures in several respects, such as improving the viscosity problems and increasing the polarity (18).

Table 3: Analysis of variance test results.

	Source	Sum of Squares	df	Mean Square	F Value	*p*-value Prob > F	
TPC (mg-GAE/g-FP)	**Model**	179.74	5	35.95	47.16	< 0.0001	significant
	A-	123.14	1	123.14	161.56	< 0.0001	
	B-	0.8851	1	0.8851	1.16	0.3169	
	AB	2.00	1	2.00	2.63	0.1491	
	A²	52.04	1	52.04	68.28	< 0.0001	
	B²	2.42	1	2.42	3.17	0.1180	
	Residual	5.34	7	0.7622			
	Lack of Fit	3.18	3	1.06	1.96	0.2616	not significant
	Pure Error	2.16	4	0.5395			
	Cor Total	185.08	12				
C.V. = 8.08 %	*R^2=0.9712*		*Adjusted R^2=0.9506*			*Predicted R^2=0.8094*	
TA (mg-cyn-3-glu/ g-FP)	**Model**	2.80	5	0.5605	36.27	< 0.0001	significant
	A-	1.58	1	1.58	102.02	< 0.0001	
	B-	0.5931	1	0.5931	38.38	0.0004	
	AB	0.0169	1	0.0169	1.09	0.3310	
	A²	0.6145	1	0.6145	39.76	0.0004	
	B²	0.0709	1	0.0709	4.59	0.0694	
	Residual	0.1082	7	0.0155			
	Lack of Fit	0.0677	3	0.0226	2.23	0.2271	not significant
	Pure Error	0.0405	4	0.0101			
	Cor Total	2.91	12				
C.V. = 5.31 %	*R^2=0.9628*		*Adjusted R^2=0.9363*			*Predicted R^2=0.7447*	
Cyanidin-3-glucoside (mg/g-FP)	**Model**	26.94	5	5.39	192.56	< 0.0001	significant
	A-	6.86	1	6.86	245.01	< 0.0001	
	B-	1.02	1	1.02	36.40	0.0005	
	AB	0.6791	1	0.6791	24.27	0.0017	
	A²	14.35	1	14.35	512.96	< 0.0001	
	B²	0.1710	1	0.1710	6.11	0.0427	
	Residual	0.1959	7	0.0280			
	Lack of Fit	0.1098	3	0.0366	1.70	0.3033	not significant
	Pure Error	0.0860	4	0.0215			
	Cor Total	27.13	12				
C.V. = 7.48 %	*R^2=0.9928*		*Adjusted R^2=0.9876*			*Predicted R^2=0.9650*	

As we already mentioned, RSM approach also provided us with optimization of a system in order to maximize the yields (19). Table 4 summarizes the optimum conditions and the maximum findings. In order to verify the results, a validation study was also performed. The difference between the actual and predicted values depending on the derived second-order models also confirms the reliability of the outcome. Figures 3a, 3b, and 3c also demonstrate the agreement of experimental and estimated results with each other for every response.

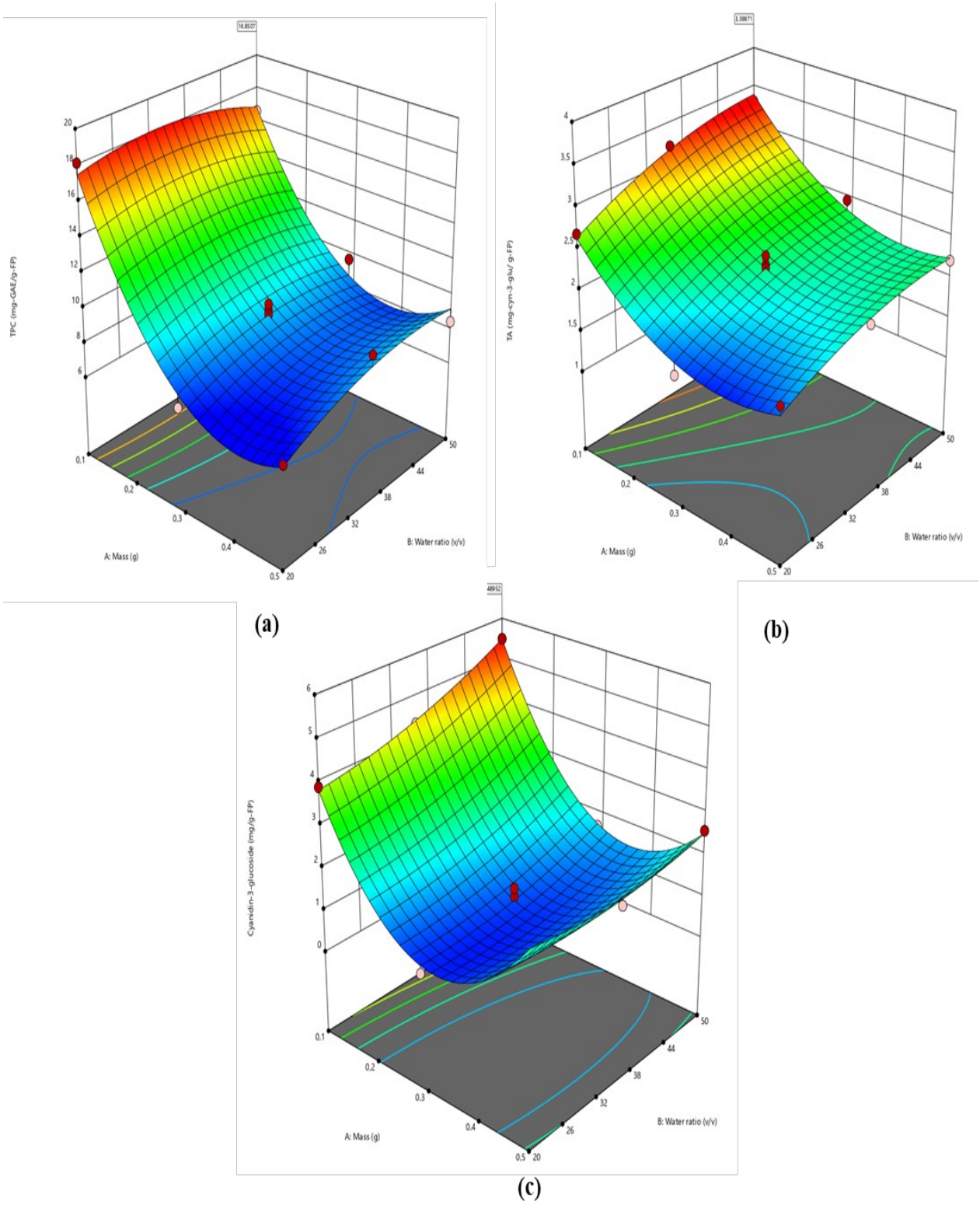


Figure 2: Response surface plot for the TPC (a), TA (b), and cyanidin-3-glucoside (c) as a function of mass to water ratio.

Table 4: Verification results of the optimum conditions for the MAE.

Optimum Extraction Conditions		Response	Predicted	Experimental	Error
A (g)	**B (%, v/v)**				
0.1	50	**TPC**	16.85	17.10	1.46%
		TA	3.39	3.45	1.74%
		Cyanidin-3-glucoside	5.49	5.52	0.54%

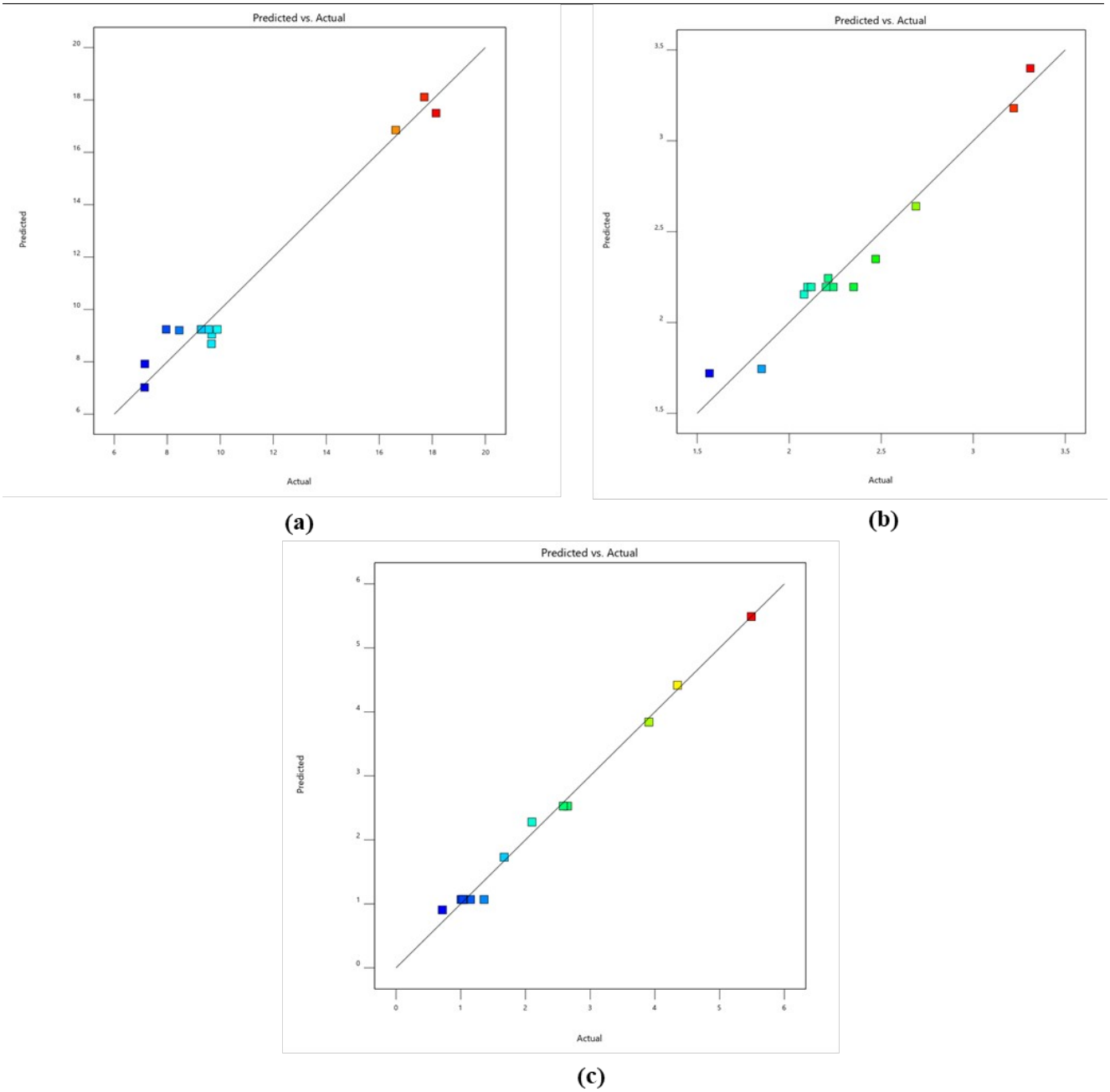
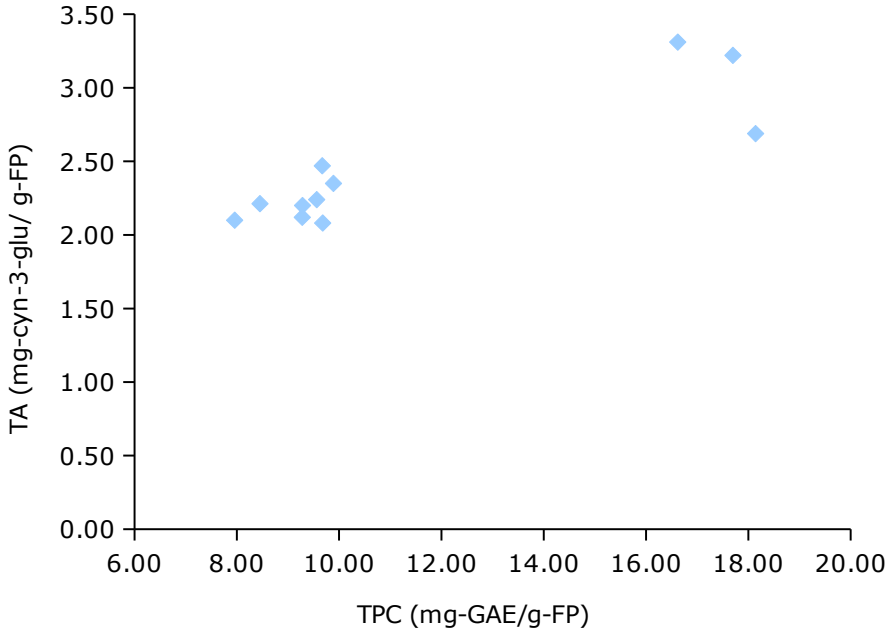


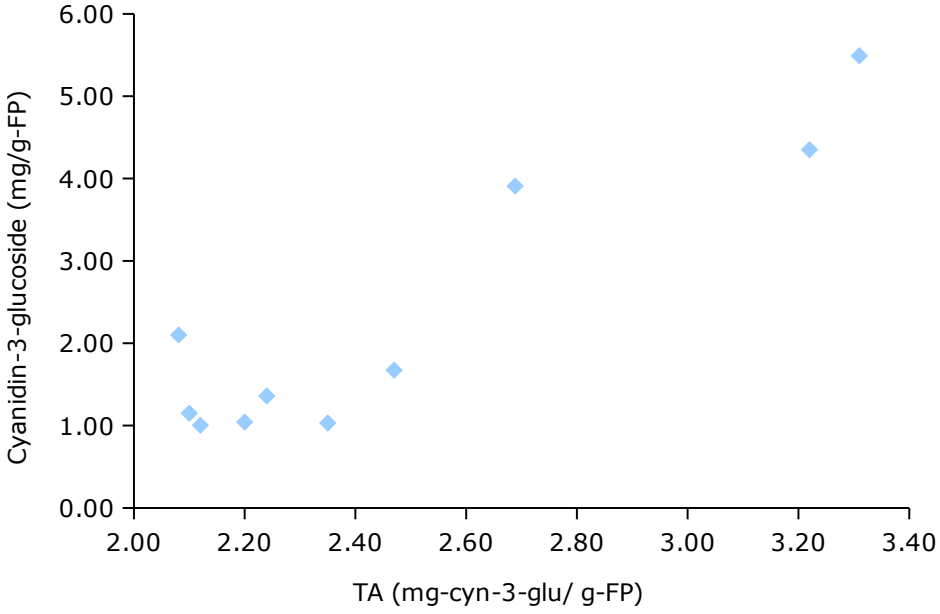
Figure 3: Actual findings versus predicted findings for TPC (a), TA (b), and cyanidin-3-glucoside (c).

Evaluation of Bioactivity Test Results

The relationship between the measured values has also been established by means of correlations (r), as seen in Figure 4. When the correlation between TPC and TA is examined (Figure 4a), it can be concluded that TA makes a significant contribution to the total amount of phenolic substances (r>0.80). Similarly, there is a strong agreement between cyanidin-3-glucoside and TPC/TA (r>0.80). As a result, cyanidin-3-glucoside seems to be one of the principal phenolic components in sour cherry peels.



(a)



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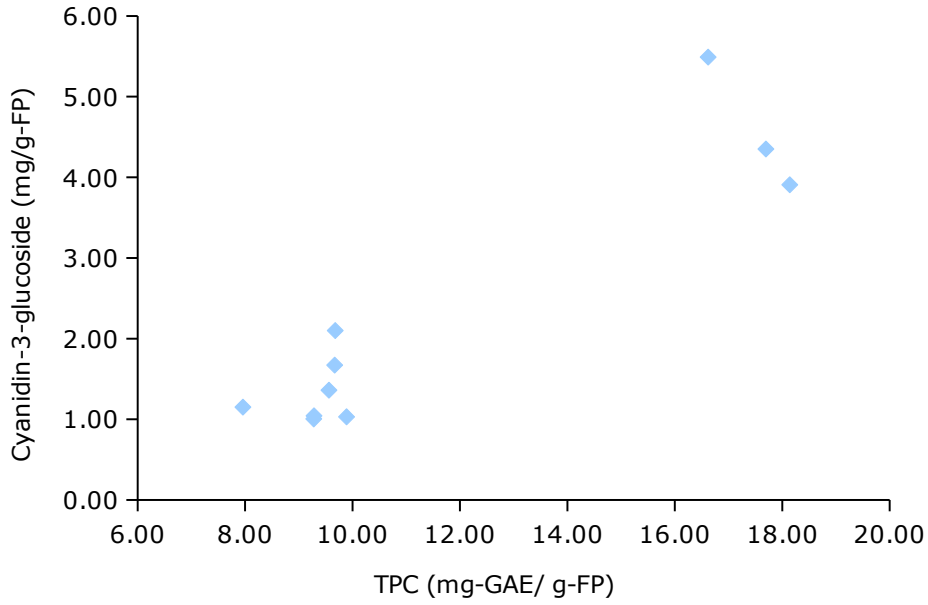

(c)

Figure 4: Correlation between TPC and TA (a), TA and cyanidin-3-glucoside (b), and TPC and cyanidin-3-glucoside (c).

CONCLUSIONS

Microwave-assisted extraction was applied to recover fine chemicals from waste by-products of sour cherry peels. The yields were evaluated in terms of total phenolic, anthocyanin, and cyanidin-3-glucoside content. Central Composite Design with the Desing Expert software has been successfully employed for the current extraction system. The suggested second-order equations are very adequate to describe the experimental data based on several statistical indicators (F-values, P-values, non-significant lack of fit, R^2, adjusted R^2, predicted R^2, and low coefficient of variation). Moreover, the relationship between anthocyanin and cyanidin-3-glucoside contents and the total phenolics of the extracts also points out that anthocyanins make a notable contribution to the total phenolics.

CONFLICT OF INTEREST

The author declares that she has no conflict of interest.

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