Ultrasound assisted extraction of bioactive substance from waste by-products of Sour cherry juice

Ebru Kurtulbaş*, Mehmet Bilgin and Selin Şahin

Istanbul University - Cerrahpaşa, Chemical Engineering Department, 34320, Avcılar, Istanbul, Turkey

e-mail:ebru.kurtulbas@istanbul.edu.tr, mbilgin@istanbul.edu.tr, selins@istanbul.edu.tr

*Corresponding Author e-mail: ebru.kurtulbas@istanbul.edu.tr

Phone: +90-212-4737070 (17989), Fax: +90-212-4737180

ABSTRACT

There are many divergent type of sour cherries worldwide, including Europe, North America and Asia and their consumption considerably common. Sour cherry production continues to increase every year. It was reached 1.38 million in 2016 (Yılmaz et al., 2015). Therefore, a large amount of waste is generated. This huge amount of waste by-product should be valorized for national and environmental economy. Sour cherries contain significant level of anthocyanins with high antioxidant activity. (Blando et al., 2004).

In this study, total phenolic content (TPC) and total anthocyanins (TA) content of sour cherry residues have been evaluated systematically. Ultrasound-assisted extraction system has been used for the recovery of bioactive components. The extraction time of the system was determined before proceeding with routine studies in order to obtain an extract rich in biologically active substances from sour cherry peels with ultrasound assisted extraction. Additionally, several kinetic models (Film theory, Peleg model, first order and second order mechanism model) were employed to examine the kinetics of UAE. Face-centred composite design was carried out with three independent parameters to identify the effect on the responses. Independent variables were chosen as amplitude, solid mass and solvent concentration of ethanol-water mixture. TPC and TA were chosen as the response. The results of the present study suggest that 500 W of microwave power, 80% solvent concentration and 90 s of extraction time should be chosen as optimal operating conditions in order to extract the maximal TPM (46.52 mg-GAE g^{-1} FM) and TA (6.57 mg-cyn-3-glu g^{-1} FM).

Keywords: Ultrasound assisted extraction; sour cherry peels; anthocyanins; cyanidin-3-glucoside; response surface methodology.

1. Introduction

Phenolic compounds, represent one of the most common groups of compounds in the plant kingdom with more than 8000 structures. Phenolic compounds are characterized by having an aromatic ring bearing one or more hydroxyl (-OH) groups when examined structurally (Shetty et al., 2006). These compounds are secondary (secondary) metabolites synthesized by plants during normal development and in response to stress conditions such as infection, UV irradiation, herbivores, and reactive oxygen species. Phenolic compounds, also known as polyphenols, are important bioactive components known for their antioxidant activity and radical scavenging capacity. They have higher in vitro antioxidant capacity than other antioxidants and are the most stable and potent type of food antioxidants such as vitamins and carotenoids. In this regard, they defend other compounds or tissues from damage caused by free radicals (Martins et al., 2011).

Sour cherry is at the top of the list of fruits considered as "super foods" due to its health-beneficial properties, especially in the United States of America in the last 15 years. Sour cherry contains a lower amount of simple sugar compared to cherries. Likewise, it exhibits higher vitamin A and β -carotene contents and higher total phenolic content than cherry fruit. In addition, it has been reported in some articles that sour cherry fruit contains higher anthocyanins compared to cherry fruit. Studies have reported that high levels of polymeric procyanidins detected in sour cherry varieties contribute to their high antioxidant activities (Blando and Oomah, 2019). Sour cherry is recognized as an excellent source of phenolic compounds and polyphenols. The polyphenol composition of the sour cherry fruit has been studied in various studies. The finding that sour cherries contain significant levels of anthocyanins has drawn attention to this species (Wang et al., 1997). The three main phenolic substances found in sour cherries are anthocyanins, flavan-3-ols and hydroxycinnamic acids.

Ultrasound is one of the key technologies in achieving the goal of sustainable "green" chemistry and extraction. It is well known that ultrasound has a significant impact on the yield of various processes in the chemical and food industry. Extractions can be completed in minutes with high repeatability, reducing solvent consumption, simplifying operation and operation, ensuring higher purity of the final product, consuming only part of the fossil energy required for the traditional extraction method and eliminating the final treatment of wastewater bu using ultrasound (Chemat and others, 2017a). Ultrasonically assisted extraction (UAE) is a new, green and fast developing technology suitable for increasing the extraction efficiency of bioactive compounds. Ultrasound mainly acts by generating cavitation bubbles in the biological matrix, whereby bioactive compounds have been reported to achieve high yields and extraction rates. Moreover, it provides significant economic and environmental benefits and has great potential for development-implementation work (Wen et al., 2018).

In this study, it was aimed to investigate the effect of amplitude, solid mass and EtOH solvent concentration in ultrasonically assisted extraction on the extraction efficiency of anthocyanin and

phenolic compounds in cherry by-products. Optimization of extraction system conditions was also included in this study while examining the efficiency of UAE. In addition to the experimental design, the response surface methodology (RSM) was implemented to create a mathematical model and optimize the UAE parameters. It is also necessary to understand the basic principles of the process while optimizing the system. For this reason, the ultrasonically assisted extraction kinetics have been investigated. In this way, the study is intended to contribute valuable information to the industrial-scale economic evaluation of the method.

2. Materials and Methods

2.1. Chemicals and reagents

Ethanol (99.9%) (EtOH), methanol (99.9%) (MeOH), hydrochloric acid (HCl), sodium carbonate (Na_2CO_3) and Folin-Ciocalteu reagent used in the study were obtained from Merck (Darmstadt, Germany). Gallic acid, cyanidin-3-glucoside, potassium chloride, sodium acetate, were obtained from Sigma-Aldrich (St., MO, USA). Using Millipore, Milli-Q pure water systems, deionized water and ultrapure water (18 ohm) were supplied.

2.2.Plant material

In May 2019, the by-products of sour cherry juice are provided from Aroma Bursa Fruit Juices ve Gıda San. A.Ş. the sour cherry samples were hand pitted, and peels were separated and stored at -25 ° C until use.

2.3.Ultrasound assisted extraction

An ultrasonicator (Vibra Cell, model VCX 750, Sonics & Materials, Inc., Newtown, CT, USA) was used to perform ultrasonically assisted extraction studies (Figure 1). Pulse mode (3/7, on / off) has been applied to prevent overheating problems caused by overexposure to ultrasound. While the samples were treated with ultrasonic waves for 3 seconds at a frequency of 20 kHz, the ultrasound effect was stopped for 7 seconds during extraction. According to preliminary tests, 0.1-0.5 g of fresh cherry by-products were taken into 50 mL plastic centrifuge tubes. Then 35 mL of extraction solvent (different ratio EtOH: H_2O; 0.01% HCl) was added. The extraction time was determined as 15 minutes by kinetic studies. Trials were carried out between 10-50% amplitude levels. The extracts were passed through a 0.45 µm injection filter. Samples were preserved in 10 mL glass bottles.

2.4.Determination of total polyphenol content

Folin-Ciocalteau method was used to determine the total phenolic content (Malik and Bradford, 2006). This method is based on the color change caused by the reduction of Folin-Ciocalteau reagent by

phenolates produced in the presence of sodium carbonate. For this purpose, 20µL of extracts prepared were taken and 380µL of pure water was added on it. Then, 2000µL of Folin-Ciocalteau reagent (10%, v / v) and 1600µL of sodium carbonate (7.5%, w / v) solution were added and mixed in an ultrasonic water bath. After the samples were kept in the dark for 30 minutes, their absorbance values were recorded at 765 nm in the UV spectrophotometer (PG Instruments, T60 / Leicestershire and England). While preparing the calibration curve, pure gallic acid prepared with suitable solvents was used. Results are expressed as milligram gallic acid equivalent per fresh matter (mg-GAE g^{-1} FM).

2.5.Determination of total anthocyanin content

Total anthocyanin (TA) analysis of the extracts was calculated by the pH differential method determined by Lee et al.(Lee et al., 2005). The appropriate dilution factor was determined by diluting potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M), and the dilution factor was calculated relative to the initial volume. Absorbance were measured at the wavelengths of 520 and 700 nm (Mónica Giusti and Wrolstad, 2005). Results are expressed as the equivalent of mg cyanidin-3-glucoside substance (mg-cyn-3-glu g^{-1} FM) per gram fresh matter. The absorbance of the diluted samples was calculated according to Equation (1), and the total anthocyanin value was calculated according to Equation (2). For cyanidin-3-glucoside with a molecular mass of 449.2 $gmol^{-1}$, the molar absorptivity was accepted as 26,900 L mol^{-1} cm^{-1}.

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \quad (1)$$

$$TA = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (2)$$

2.6.Kinetic study of ultrasound assisted extraction

In the present study, pseudo-first order, pseudo-second order, film theory and Peleg's kinetic model were fitted to the experimental data for the kinetic modelling of phenolic compounds extraction by UAE process. Kinetic equations for all models are proffered in the Table 1. The kinetic parameters of the four models were designated from the TPC results of UAE. Film theory and Peleg's kinetic model were based on two-stage extraction mechanism. These models are two-parametric (Bilgin and Şahin, 2013; Segovia et al., 2016; Veličković et al., 2008). One parameter describes the washing stage (termed washing coefficient) and the second parameter identifies the slow extraction (called slow extraction coefficient).

2.7.Experimental design and statistical analysis

The response surface method (RSM) is an experimental optimization procedure based on physical experiments or computer experiments (simulations) and experimental observations. First in 1951,

G.E.P. Box and K.B. It was developed by Wilson. In most of the optimization methods, the effects of the parameters on each other are ignored and only the effects of different parameters on the results are calculated. However, the parameters often affect each other, especially in physical experiments. For example, if the parameters are density and temperature, it will not be sufficient to consider just two different effects, as temperature can also affect density. RSM is a method that aims to eliminate this disadvantage.

In this study, central composite design (CCD) design via RSM was applied with selected independent variables in order to explore the effect of variables on response variables (Table 2). For this purpose, Design-Expert (Statease, Minneapolis, MN, USA) software (version 12.0) was used. In the response surface method, a function has been obtained for the estimation of the results. These functions are expressed by the Equation (3):

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i x_i+\sum_{i=1}^{k}\beta_{ii}x_i^2+\sum_{i=1}^{k-1}\sum_{j=i+1}^{k}\beta_{ij}x_i x_j+\varepsilon \quad (3)$$

In the equation Y; It refers to the response variable for RSM. β_0; constant, β_i, linear impact coefficient, β_{ii}, quadratic force impact coefficient, β_{ij} (i and j = 4); interaction coefficient and X_i (i = 1-4); refers to the uncoded factor.

Analysis of variance (ANOVA) was performed with the same software in order to verify the model determined by the experimental design program and to examine the interactions of the variables among themselves. In addition, all trials were performed in triplicate in order to obtain reliable data and statistically analyzed with InStat® software (GraphPad, San Diego, CA, USA) using these means and standard deviation data. The significance of the changes between parameters was determined by means of the Tukey test. The correlation between the experimental and the calculated data was estimated by the correlation coefficient (r) and the root-mean-square deviations (rmsd) considering the Equation (4):

$$rmsd=\sqrt{\frac{\sum_{i=1}^{n}(C_{i,exp}-C_{i,cal})^2}{n}} \quad (4)$$

In equation; *n* is the number of the repetitions, $C_{i,exp}$ is the concentration of experiment *i* and $C_{i,cal}$ is the calculated concentration of the *i*.

3. Results and Discussion

3.1. Effects of independent parameter on UAE

Table 3 shows the experimental plan of the current study designed with CCD and shows the experimental results under the given conditions. As seen in Table 4, the software (Design-Expert) produced 20 experimental studies with 5 center points (30% amplitude, 0.3 grams sample and 50%

ethanol solution) for the relevant system. In addition, three-dimensional graphs (Figure 4.8-4.10) drawn with the relevant software visually present the effect of process parameters.

The total phenolic content of sample varies between 18.65 and 47.59 mg-GAE g^{-1} FM. The total anthocyanin content of the sour cherry peel extracts obtained with UDE was between 2.78 and 7.30 mg-cyn-3-glu g^{-1} FM. Figure 2 shows the effects of amplitude value and solids content on total phenolic content (a) and total anthocyanin (b) at constant EtOH concentration (80%, v / v).

It is seen that the amplitude increase in the extraction of cherry peels with the UDE method has a positive effect especially in terms of TPC (Figure 2). This is an expected result because increasing energy increases the extraction of polyphenols as it is a situation that supports diffusion. A similar result was observed in the extraction of phenolic compounds with UDE from hibiscus flower (Şahin et al., 2020). Considering the effect of the amount of solid matter, it can be said that it has a very mild effect for TA. If evaluated in terms of TPC, an effect such as a decrease and then an increase is observed. This finding can be explained by the fact that increasing the sample mass decreases the surface area. Increasing the sample mass reduces the surface area available for the solvent to penetrate the sample matrix and dissolve phenolics, thus causing a reduction in the extraction efficiency of these target components (Şahin, 2015).

Figure 3 shows the effects of amplitude value and EtOH solvent concentration on total phenolic substance (a) and total anthocyanin (b) at constant solids (0.1 g).

Solvent concentration had a similar effect on both systems. The increase in alcohol ratio in the aqueous alcohol system regularly increased the TPC and TA yields. In addition to the explanations made above, we can explain the success of the water-EtOH couple as follows: Water acts as a swelling agent for plant material, ethanol breaks the bonds between solutes and plant matrices, in favor of extraction yield (Ilbay et al., 2013).

Figure 4 shows the effects of solid matter content and EtOH solvent concentration at constant amplitude value (34.34%) on total phenolic substance (a) and total anthocyanin (b). The effects of solids content and solvent concentration on the UDE system are shown in Figure 3.

3.2. Model fitting

ANOVA table was produced while obtaining TPC and TA-rich extract from sour cherry peels with the UAE method. Table 5 shows the ANOVA test results calculated for TPC and TA extraction from sour cherry peels with UAE using CCD. Second order polynomial equations produced for both answer values were found to be statistically significant ($p < 0.0001$).

Equations (4) and (5) give the last equations derived by Design-Expert in terms of factors coded for TPC and TA, respectively:

TPC (mg-GAE g^{-1} FM)	$28.83 + 4.73\,A - 5.74\,B + 3.16\,C - 0.5653\,AB + 1.17\,AC - 2.45\,BC - 3.57\,A^2 + 5.37\,B^2 + 0.8045\,C^2$	(4)
TA (mg-cyn-3-glu g^{-1} FM)	$5.99 - 0.5318\,A + 0.4838\,B + 0.3397\,C + 0.4206\,AB + 0.4479\,AC - 0.7797\,BC - 0.0782\,A^2 - 0.1167\,B^2 + 0.0391\,C^2$	(5)

The independent parameters of the extraction process were found to be important for the system ($p < 0.05$). As seen in Table 5, the interactions were not found to be statistically significant except for the interaction between solvent concentration and solids content for TPC ($p > 0.05$). In addition, the second-order forces of the other parameters were not found to be effective, except for the second-order power of EtOH concentration. All independent variable interactions are observed to be effective ($p < 0.05$) when ANOVA results are examined in terms of TA (Table 4). Second-order powers of the independent variables were not found to be statistically effective for the TA system ($p > 0.05$).

On the other hand, the lack of fit value for both answer values was calculated to be greater than 0.05, and this situation supports that the models used are suitable for the data.

It can be said that there is a satisfactory relationship between the experimental data and the estimated data for both systems (TPC and TA) based on the ANOVA findings (Figure 5).

3.3. Optimization and validation studies

Table 6 gives the best UAE process conditions required to obtain the highest TPC (47.59 mg-GAE g^{-1} FM) and TA (6.63 mg-cyn-3-glu g^{-1} FM) yields according to the optimization study of RSM with Desing-Expert software. Verification of the conditions has also been tested. The fact that the residual between the experimental and the calculated results support that the model is suitable for the systems.

3.4. Kinetic Analysis

Figure 6 shows that the change of TPC od sour cherry peels extract with time. It can be seen from figure TPC of extract is reached balance in between fifteenth - eighteenth minute of extraction. Furthermore kinetic parameters, correlation coefficient of kinetic models, calculated from equations presented in the Table 1, are demonstrated Table 6. To analyze the experimental verification with the model prediction in total phenolic content of sour cherry peels via UAE, the correlation between the experimental and calculated values was proved by root mean squared deviation. In agreement with a high correlation coefficient (0.81 - 0.89) and low root mean square deviation (2.21 - 13.64) are considered, it is seen that the kinetics of the sour cherry peels extraction process performed with UAE are suitable for the Peleg's model. The estimated parameters of Peleg's constants, termed k_1 and k_2, and values of B_0, R^2, *rmsd* are exhibited in Table 3. It should be remarked that a lower k_1 value indicates a faster rate of the process, whilst the lower k_2 value symbolizes a higher extraction level.

A similar result was observed at the research of Kadam et al. (Kadam et al., 2015) about extraction kinetics of bioactives from brown seaweed (*Ascophyllum nodosum*). Furthermore, a similar result was observed in research investigating lycopene extraction from tomato processing wastes. First order kinetic model, mass transfer model and Peleg's model were applied while exploring the extraction kinetic. Peleg's model showed the most suitable model for experimental data by expressing the highest R^2 and lowest *rmsd* values, when the results are examined (Poojary and Passamonti, 2015).

4. Conclusion

The present research has reviewed the optimized ultrasound-assisted extraction process and kinetic model for sour cherry peel extraction. Investigated samples were found to be remarkably rich in phenolic and anthocyanin content. The research has proposed that 34.34 % amplitude, 0.1 g solid mass and 80% solvent concentration as optimum process parameters to achieveTPC (49.90 mg GAE g^{-1} FM) and TA (6.57 mg cyn-3-glu g^{-1} FM) from sour cherry peel. The suggested conditions have been confirmed by a verification study, where the differentiation between the experimental and calculated data was about 2.76%. The kinetics of the extraction of the phenolic compounds from the sour cherry peels by means of UAE was reported by Peleg's model. Modeling of the extraction kinetics will provide not only theoretical knowledge of the process but also its usefulness for process operation.

Conflict of Interest

The authors declare that there is no conflict of interest in writing upon submission of the manuscript.

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Table 1. Assessment of kinetic models of phenolic compounds extraction by UAE from sour cherry peels.

Model	Kinetic Equation	Linearized Form
Pseudo first-order	$C = (C_e - C_o)[(1 - e^{-k_1 t})] + C_o$	$\ln\left(\frac{C_e}{C_e - C_t}\right) = k_1 t + \ln\left(\frac{C_e}{C_e - C_o}\right)$
Pseudo second-order	$\frac{dC_t}{dt} = k_2 (C_e - C_t)^2$	$\frac{t}{C_t} = \frac{1}{k_2 C_e^2} + \frac{t}{C_e}$
Peleg's model	$C = C_o + \frac{t}{k_3 + k_4 t}$	$C_t = \frac{t}{k_3 + k_4 t}$
Film Theory	$\frac{C_t}{C_e} = 1 - (1 - b)e^{-k_5 t}$	$\ln\left(1 - \frac{C_t}{C_e}\right) = \ln(1 - b) - k_5 t$

Notation: C_t –the concentration of TPC in sour cherry peels extract at time t (min), mg-GAE L^{-1}; C_e– maximum concentration of TPC in sour cherry peels extract, mg-GAE L^{-1}; C_o– initial concentration of TPC in sour cherry peels extract, mg-GAE L^{-1}; t – time, min; k_1 – first order rate constant, min^{-1}; k_2– second order rate constant, L mg-1 min^{-1}; k_3– Peleg's rate constant at the beginning, min g mg^{-1} GAE; k_4– Peleg's capacity constant, mg GAE g^{-1} min^{-1};b– washing coefficient according to film model, 1; k_5– slow extraction coefficient according to film model, min^{-1}.

Table 2. Summary of the ultrasound assisted extraction parameters with their units, symbols and levels.

Process Parameters	Units	Symbol of the Parameters	Levels with the Codes		
			−1	**0**	**1**
Amplitude	%	A	10	30	50
Solid Mass	g	B	0.1	0.3	0.5
Solvent Concentration	%, v/v	C	20	50	80

Table 3. Experimental results for the TPC and TA extraction depending on CCD for independent parameters.

Experiment No	Amplitude (%)	Solid Mass (g)	Solvent Concentration (%, v/v)	TPC (mg-GAE/ g-FM)	TA (mg-cyn-3-glu/ g-FM)
1	50	0.1	80	47.59±0.001[a]	6.00±0.002[a]
2	30	0.3	50	30.39±0.008[b]	6.54±0.001[b]
3	30	0.3	50	30.39±0.008[b]	5.90±0.003[c]
4	10	0.3	50	18.65±0.002[c]	6.37±0.002[d]
5	30	0.3	50	25.36±0.003[d]	5.83±0.001[e]
6	50	0.3	50	31.93±0.004[e]	5.38±0.001[f]
7	50	0.1	20	35.73±0.005[f]	2.78±0.003[g]
8	30	0.1	50	41.25±0.001[g]	5.38±0.001[f]
9	30	0.3	50	28.25±0.002[h]	6.02±0.000[h]
10	50	0.5	80	31.55±0.001[i]	6.13±0.001[i]

11	10	0.1	80	36.46±0.003^{j}	6.90±0.005^{j}
12	30	0.3	50	28.69±0.006^{k}	6.02±0.002^{h}
13	10	0.5	20	23.65±0.003^{l}	7.30±0.001^{k}
14	30	0.3	20	24.77±0.001^{m}	5.56±0.003^{l}
15	50	0.5	20	27.83±0.005^{n}	6.29±0.001^{m}
16	10	0.5	80	21.00±0.001^{o}	5.61±0.005^{n}
17	30	0.3	80	34.56±0.002^{p}	6.42±0.006^{o}
18	10	0.1	20	27.61±0.003^{r}	5.73±0.001^{p}
19	30	0.3	50	29.78±0.001^{s}	5.76±0.001^{r}
20	30	0.5	50	27.21±0.001^{t}	6.29±0.006^{m}

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

Table 4. Analysis of variance test results for the TPC and TA extraction depending on CCD.

TPC	**Sum of Squares**	**df**	**Mean Square**	**F Value**	***p*-value**	
Model	819.00	9	91.00	22.51	< 0.0001	significant
A-Amplitude	223.32	1	223.32	55.24	< 0.0001	
B-Solid mass	329.49	1	329.49	81.51	< 0.0001	
C-Solvent Concentration	99.68	1	99.68	24.66	0.0006	
AB	2.56	1	2.56	0.6323	0.4450	
AC	11.03	1	11.03	2.73	0.1296	
BC	48.16	1	48.16	11.91	0.0062	
A^2	35.05	1	35.05	8.67	0.0147	
B^2	79.27	1	79.27	19.61	0.0013	
C^2	1.78	1	1.78	0.4403	0.5220	
Residual	40.43	10	4.04			
Lack of Fit	22.23	5	4.45	1.22	0.4155	non-significant
Pure Error	18.19	5	3.64			
TA	**Sum of Squares**	**df**	Mean **Square**	**F Value**	***p*-value**	
Model	14.32	9	1.59	34.93	< 0.0001	significant
A-Amplitude	2.83	1	2.83	62.08	< 0.0001	
B-Solid mass	2.34	1	2.34	51.39	< 0.0001	

C-Solvent Concentration	1.15	1	1.15	25.33	0.0005	
AB	1.42	1	1.42	31.06	0.0002	
AC	1.60	1	1.60	35.23	0.0001	
BC	4.86	1	4.86	106.78	< 0.0001	
A^2	0.0168	1	0.0168	0.3695	0.5568	
B^2	0.0374	1	0.0374	0.8220	0.3859	
C^2	0.0042	1	0.0042	0.0925	0.7673	
Residual	0.4555	10	0.0456			
Lack of Fit	0.0718	5	0.0144	0.1872	0.9552	non-significant
Pure Error	0.3837	5	0.0767			

Table 5. Verification results of the optimum conditions for the UAE of sour cherry peel.

Optimum Extraction Conditions						
A (%)	**B (g)**	**C (%, v/v)**	**Parameter**	**Predicted**	**Experimental**	**Error**
34.34	0.1	80	**TPC**	47.59	49.90±0.002	4.62
			TA	6.63	6.57±0.001	0.91

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

Table 6. Calculated parameters of the kinetic models for UAE of total phenolic components from sour cherry peels.

Model	**Parameter**			
Pseudo first-order	k_1 (min^{-1})		R^2	*rmsd*
	0.0577		0.8949	13.64
Pseudo second-order	k_2 (L mg^{-1} min^{-1})		R^2	*rmsd*
	0.0075		0.8221	7.76
Peleg's model	k_3 (min g mg^{-1})	k_4 (g mg^{-1})	R^2	*rmsd*
	0.661	0.0801	0.8349	2.21
Film theory	*b*	k_5 (min^{-1})	R^2	*rmsd*
	0.0491	0.0185	0.8174	5.18

Figure Captions

Figure 1. Ultrasound assisted extraction system for the extraction of sour cherry peels.

Figure 2. The 3D surface plot for the (**a**) TPC, (**b**) TA of the sour cherry peels extract as a function of solid mass to amplitude (EtOH solvent concentration=80.00 %).

Figure 3. The 3D surface plot for the (**a**) TPC, (**b**) TA of the sour cherry peels extract as a function of EtOH solvent concentration to amplitude (Solid mass= 0.1g).

Figure 4. The 3D surface plot for the (**a**) TPC, (**b**) TA of the sour cherry peels extract as a function of EtOH solvent concentration to solid mass (Amplitude=34.34 %).

Figure 5. The relationship between the experimentally achieved values of the TPC and TA of UAE method versus the calculated values using the quadratic equations of Design Expert 12.0.1.

Figure 6. Determination of extraction time in UAE method applied to cherry peels.

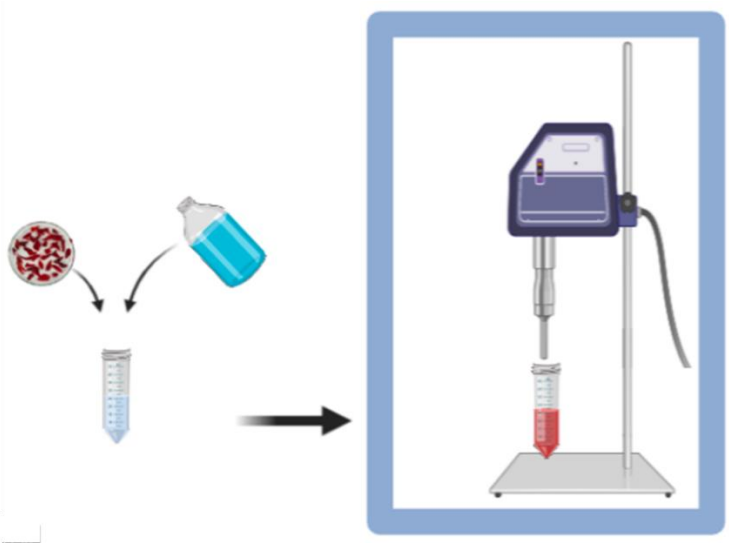

Fig 1.

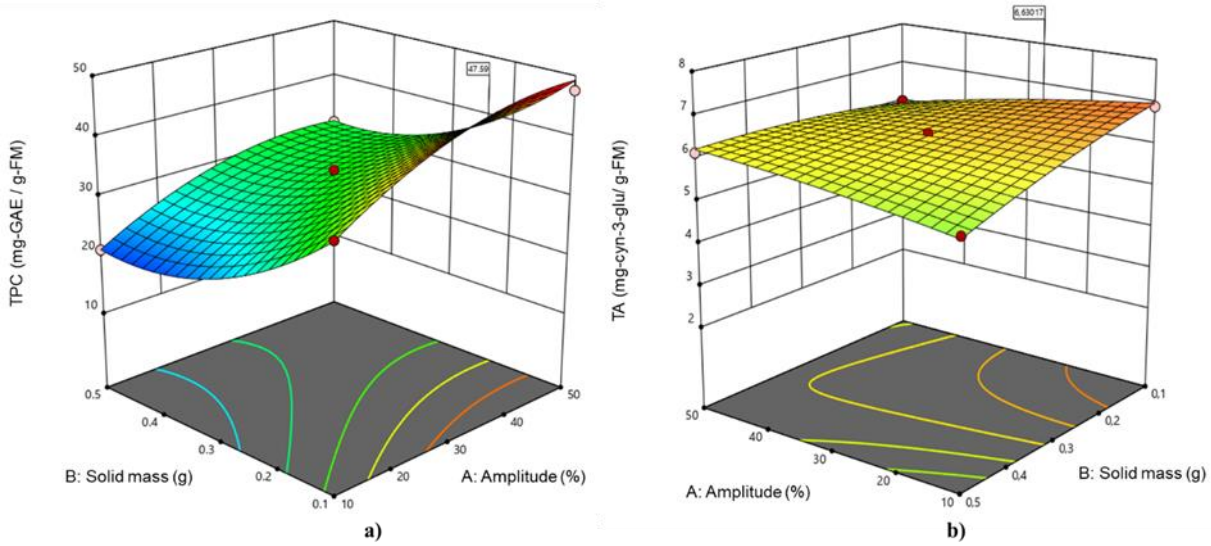
TPC (mg-GAE / g-FM)
50
40
30
20
10
47.59
0.5
0.4
0.3
0.2
0.1
B: Solid mass (g)
10
20
30
40
50
A: Amplitude (%)
a)
TA (mg-cyn-3-glu/ g-FM)
8
7
6
5
4
3
2
6.63017
50
40
30
20
10
A: Amplitude (%)
0.5
0.4
0.3
0.2
0.1
B: Solid mass (g)
b)

Fig 2.

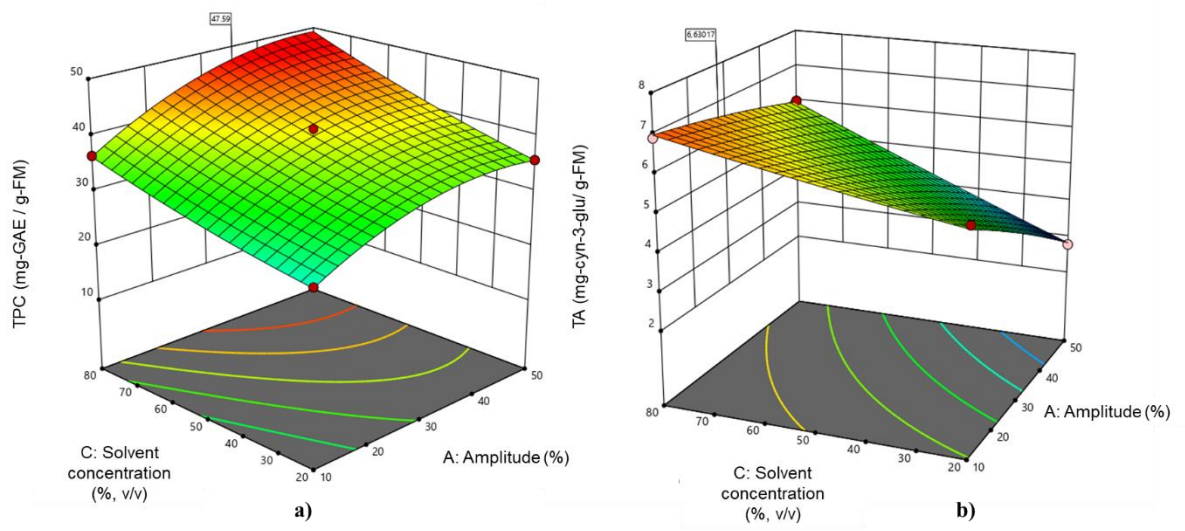
TPC (mg-GAE / g-FM)
47.59
50
40
30
20
10
80
70
60
50
40
30
20
C: Solvent concentration (%, v/v)
10
20
30
40
50
A: Amplitude (%)
a)
TA (mg-cyn-3-glu/ g-FM)
6.63017
8
7
6
5
4
3
2
80
70
60
50
40
30
20
C: Solvent concentration (%, v/v)
10
20
30
40
50
A: Amplitude (%)
b)

Fig 3.

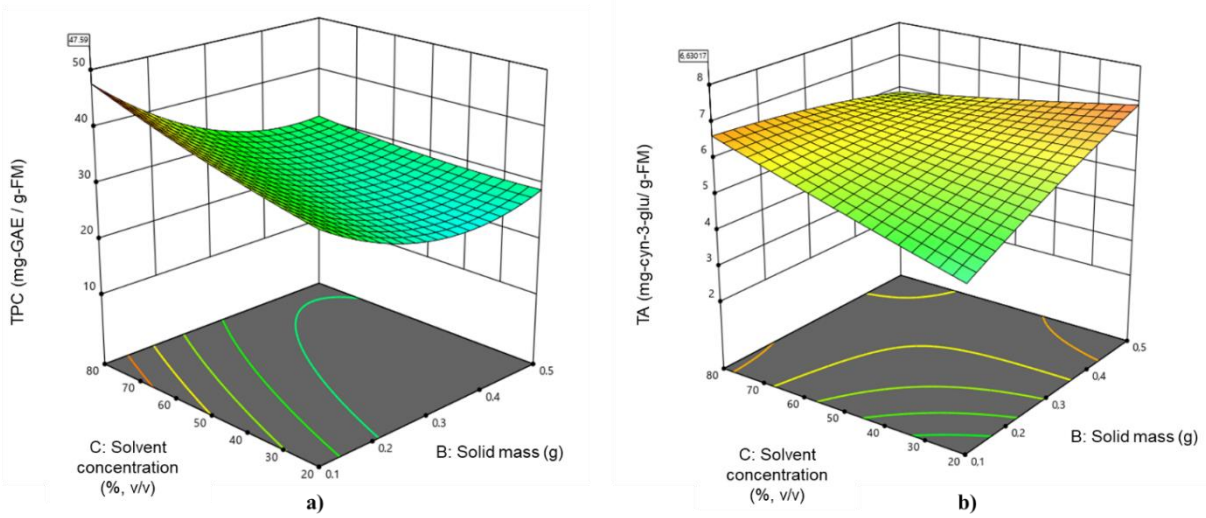
TPC (mg-GAE / g-FM)
47.59
50
40
30
20
10
80
70
60
50
40
30
20
C: Solvent concentration (%, v/v)
0.1
0.2
0.3
0.4
0.5
B: Solid mass (g)
a)
TA (mg-cyn-3-glu/ g-FM)
6.63017
8
7
6
5
4
3
2
80
70
60
50
40
30
20
C: Solvent concentration (%, v/v)
0.1
0.2
0.3
0.4
0.5
B: Solid mass (g)
b)

Fig 4.

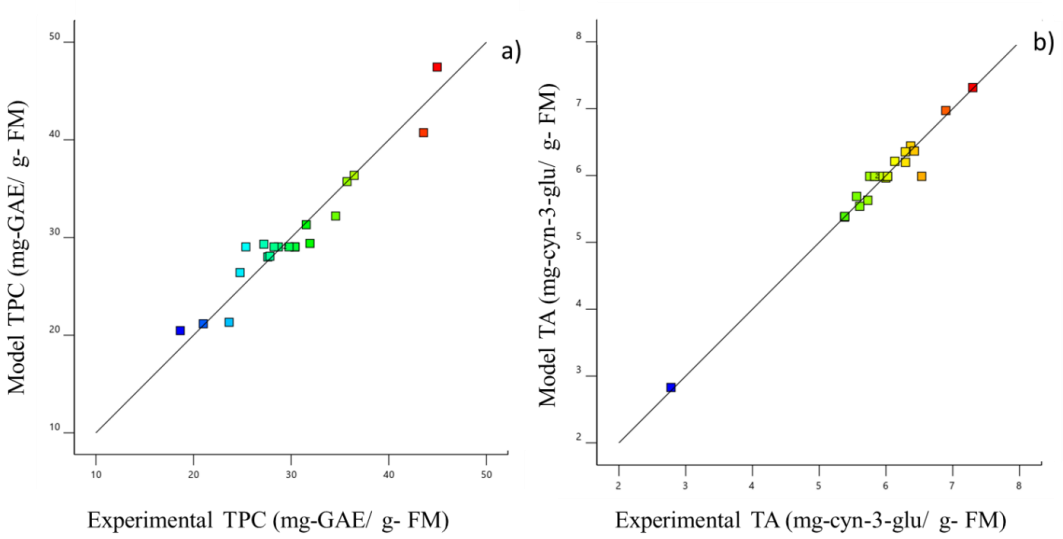
a)
Model TPC (mg-GAE/ g- FM)
Experimental TPC (mg-GAE/ g- FM)
b)
Model TA (mg-cyn-3-glu/ g- FM)
Experimental TA (mg-cyn-3-glu/ g- FM)

Fig 5.

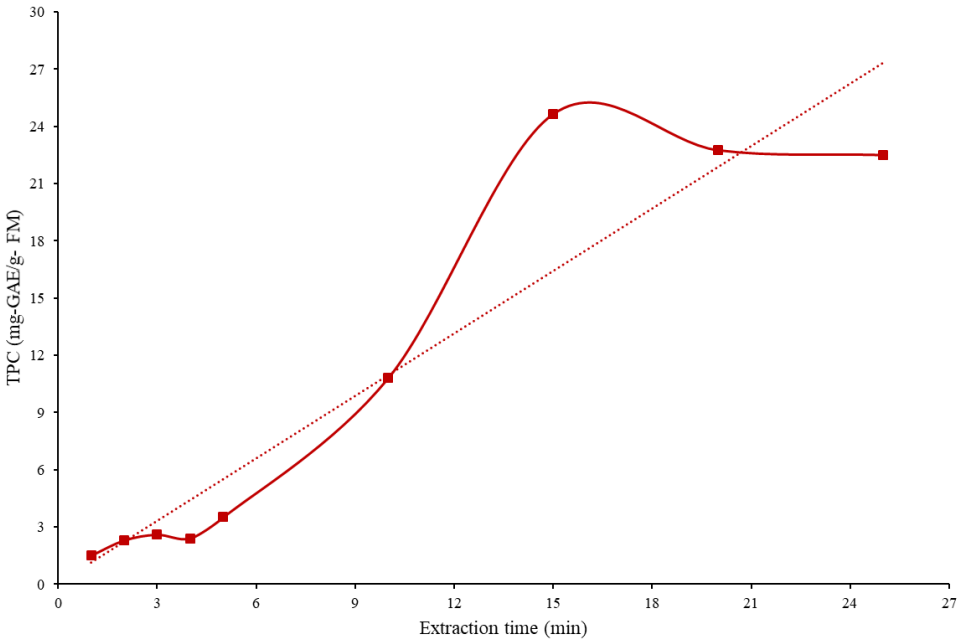
TPC (mg-GAE/g- FM)
Extraction time (min)

Fig 6.