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Intensified extraction of anthocyanins from *Berberis vulgaris* L. by pulsed electric field, vacuum-cold plasma, and enzymatic pretreatments: modeling and optimization

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Abstract

Background *Berberis vulgaris* L. is a valuable source of natural antioxidants, polyphenols, and anthocyanins compounds. Advanced extraction methods can increase extraction efficiency. This study investigated the efficiency of pulsed electric field, vacuum-cold plasma, and enzymatic pretreatment for anthocyanins extraction of *Berberis vulgaris* L.

Results Total polyphenols (TP), total anthocyanin (TA), and physicochemical properties of *Berberis vulgaris* L. were investigated. The pulsed electric field at three levels of electric intensity (3000, 5000, and 7000 V/cm) and three pulse numbers (50, 75, and 100) were applied. 7000 V/cm with the pulse number of 100 was the best condition for anthocyanin extraction (amounts of anthocyanin and polyphenol extraction were 260.28 mg/L and 462.75 mg/L, respectively). The vacuum-cold plasma was carried out at the power of 60, 70, and 80 w at different times (1, 3, and 5 min). The optimum conditions for vacuum-cold plasma were 80 w for 5 min, and anthocyanin and polyphenol amounts were 256.32 mg/L and 433.71 mg/L, respectively. The optimal conditions of enzymatic pretreatment for the maximum yield were 1.5% enzyme concentration at 60 °C (the values of extracted anthocyanin and polyphenol were 279.64 mg/L and 484.93 mg/L, respectively).

Conclusions Different extractionpretreatments demonstrated that the enzymatic pretreatment resulted in the highest extraction of anthocyanins and polyphenols from *Berberis vulgaris* L. Therefore, pectinase can act as a potential assisted extraction for the extraction process.

Highlights

- Pulsed electric field, cold plasma, and pectinase were used for Barbery anthocyanins extraction.
- Vacuum-cold plasma as novel assisted extraction technology improved anthocyanins extraction.
- Pulsed electric field enhanced anthocyanins extraction efficiency of *Berberis vulgaris* L.
- The enzymatic pretreatment appears as the best pretreatment prior to anthocyanin extraction.

Keywords *Berberis vulgaris* L. Anthocyanins, Extraction, Vacuum-cold plasma, Pulsed electric field, Pectinase

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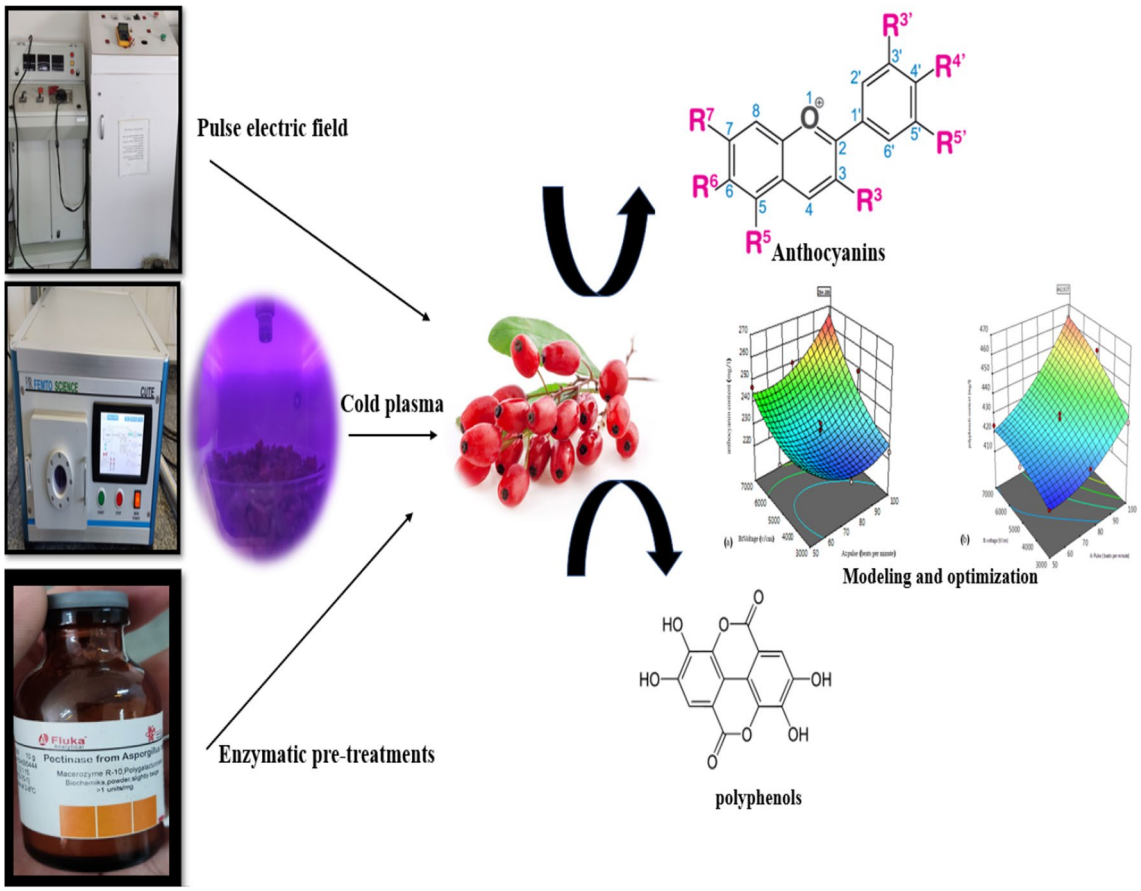
Graphical Abstract



Introduction

Berberis vulgaris L. (Barberry), known as "Zereshk" in Persian, is one of the most important medicinal plants grown in North Africa, Europe, Asia, and especially Iran. *Berberis vulgaris* L. has valuable vitamins, pigments, flavonoids, polyphenols, and anthocyanins [1]. The Phytochemicals of Berberis species are widely applied in pharmaceuticals and dietary supplements [2]. Indeed, they are used in the food industry as food additives, flavorings, and preservatives [3–5]. Previous studies reported that extraction methods influenced phytochemical components of *Berberis vulgaris* L., such as antioxidants, phenolic, and anthocyanin [6]. Therefore, it is essential to use the best processing methods to preserve the nutritional and functional potential of *Berberis vulgaris* L. Traditional methods can be simple but ineffective due to heat-sensitive bioactive compounds breaking down [7].Conventional extraction processes are associated with a high solvent requirement and low mass transfer [8, 9]. These extraction methods are labor-intensive, often expensive, and environmentally unfriendly. Therefore, it is important to find more sustainable alternative extraction techniques [10], such as supercritical fluid extraction, pulsed electric field, and cold plasma. Also, these methods are known as non-thermal or green technologies and can increase the efficiency extraction of bioactive compounds and reduce the energy consumption and extraction time [11].

Pulsed electric field (PEF) is a novel food processing technique [12]. It is a non-thermal extraction technique in which the electric field penetrates the cell wall and permeabilizes the cell membrane, causing leakage of intracellular compounds [13], which is related to a phenomenon called electroporation [14]. Indeed, a strong electric field permeabilizes the cell membrane, allowing ions and macromolecules to exit the cell [15,

16]. The parameters such as field strength, temperature, extraction time, and specific energy affect the efficiency of this extraction method [15]. Also, PEF can reduce the microbial level, retain the nutritional attributes of juices, and inactivate spoilage enzymes [17]. Pulsed electric field increased extraction yield of orange, pomelo, and lemon juices [14].

Plasma with relatively low ionization is used to scrape plant tissue and extract its active substances, called cold plasma [18]. Cold plasma is another innovative but largely unexplored approach in the extraction process. The appropriate conditions (input power, gas, and treatment time) are applied to reduce the treatment time. So, these methods are much more rapid than conventional methods [19, 20]. Keshavarzi et al. [21] investigated the enhancement of polyphenolic extraction rate with maximal antioxidant activity from green tea leaves by cold plasma. They showed that after cold plasma processing at 15 W for 15 min, total phenolic content, and antioxidant activity of green tea increased by 41.14% and 41.06%, respectively. Also, the catechin increased by 103.12% [21].

Enzymatic extraction is an alternative method that requires fewer solvents and short extraction times. It can also increase the extraction yield of active ingredients [22]. Furthermore, extractions of plant anthocyanins and vacuolar pigments with solvents are often only accomplished when the solvent is distributed in the substrate [23, 24]. Pectinases are a class of enzymes that catalyze the degradation of pectin substances [25]. Gamage et al. [26] investigated the pectinase-assisted extraction of anthocyanins from blue pea flowers, which significantly increased the total anthocyanin content of the extract [26].

Classical solvent extraction method and modern extraction methods, including supercritical carbon dioxide, high voltage electric discharge process, ultrasonic, microwave, and subcritical water, were used to extract effective compounds from *Berberis vulgaris* L. [27]. However, there were no reports about the applications of pulsed electric fields, cold plasma, and biological methods for barberry anthocyanin extraction. Therefore, this study aimed to enhance the extraction of anthocyanins from *Berberis vulgaris* L. using pulsed electric fields, vacuum-cold plasma, and enzymatic pretreatments. Furthermore, the study aimed to establish a relationship between these methods to enhance the efficiency of extracting anthocyanins and polyphenol contents from *Berberis vulgaris* L. The study employed response surface methodology (RSM) based on a central composite design (CCD) to optimize the extraction of anthocyanins and polyphenols from *Berberis vulgaris* L. In the subsequent step, the results under the optimum conditions were compared to select the most effective method for extracting barberry anthocyanins.

Material and methods

Materials

Fresh *Berberis vulgaris* L. was picked manually in Mashhad (October 2022) (Khorasan Razavi, Iran). Reagent 2-diphenyl-1–1-picrylhydrazyl (DPPH) and Folin–Ciocalteu reagent were prepared from Sigma (Sternheim, Germany). Potassium chloride (KCl), sodium acetate (CH_3COONa), and gallic acid were prepared from Merck Company (Darmstadt, Germany). Pectinase (Polygalacturonase) was prepared from FLUKA. Distilled water, other reagents, and chemicals were analytical grades and used without further purification. All other chemicals and solvents were analytical grade and obtained from Merck (Darmstadt, Germany).

Physicochemical properties of Berberis vulgaris L.

The moisture content of the samples was obtained according to AOAC (1998, 2016) method by drying the samples in an oven (UF55 MEMMERT) at 105 °C until reaching a constant weight. The ash, fat, reducing sugars, dietary fiber, and protein contents were evaluated [28].

Solvent extraction procedure

Exactly 10.0 g of *Berberis vulgaris* L. was mixed with 40 mL of acidic ethanol solution with HCl (15:85 v/v) and stirred for two h at 30 °C. After the extraction time, the mixture was filtered using Whatman filter paper (No. 4), and the solution under the filter was kept at 4 °C until the measurement time. Total anthocyanin and total polyphenol contents were determined in the obtained extract [29].

Optimization of pretreatment methods conditions

In order to compare the changes in extraction efficiency with the conventional solvent extraction methods, pulsed electric field, plasma, and enzymatic pretreatment methods were used. A certain amount of *Berberis vulgaris* L. sample was weighed and mixed with a certain amount of ethanol, and the pretreatments of the pulsed electric field method were applied to the samples. Response surface method and central composite design were used to optimize the process parameters. *Berberis vulgaris* L. was placed in a special tank at room temperature. This chamber was made of Plexiglas with dimensions of 10 cm × 10 cm square, and the distance between two electrodes (stainless steel) was 4 cm. Electric energy was transferred by a direct current to a series of capacitors, and the stored energy was discharged to the electrodes and purification chamber by a pulse switch. In this process, an 8-microfarad capacitor was used. The ability

to produce electricity by the generator was from 62.5 to 1250 V/cm. Pretreatment variables for pulsed electric fields include the number of pulses (50–100), and the voltage (4000–7000 V). The device used for this research had logarithmic pulses, so the length factor and the pulse width are not defined for this device, and the frequency was considered 1 Hz, and the distance between the two electrodes was 4 cm. The responses were total anthocyanin and total polyphenol content. PEF was designed in the central laboratory of the research institute of food science and Technology, Mashhad, Iran. [30]. Cold plasma was carried out at three levels of power (60, 70, and 80 W) and three levels of time (1, 3, and 5 min), and frequency was kept constant at 50 kHz [14]. In line with this method plasma technique as a non-thermal plasma strategy was presented via vacuum-cold plasma (DBR Femto Science, South Korea) directly on the *Berberis vulgaris* L. fruit [21]. In the enzyme pretreatment method, pectinase enzyme activity was measured at first at a wavelength of 550 nm [31]. Pectinase enzyme activity 0.91 ± 0.02 U/ml was determined. Pretreatment variables include enzyme concentration (0.5–1.5 units) and process temperature (40–60 °C) [32].

Total anthocyanin content

Total anthocyanin content was determined using the pH differential method [33]. The extract was diluted: one was diluted with 0.025 M KCl buffer (pH = 1.0), and the other was diluted with sodium acetate buffer (pH = 4.5). The diluted samples were incubated at room temperature for 15 min, then the absorbance (A) of the mixtures was measured at 510 and 700 nm using a UV–Vis spectrophotometer (DR 5000™, Hach Canada) and inserted into the following Eqs. (1, 2):

Monomeric anthocyanin pigmen

$$\text{mg C3G equivalents} = \frac{A \times M_W \times D_f \times 1000}{\varepsilon \times l} \quad (1)$$

$$A = (A_{510\text{nm}} - A_{700\text{nm}})_{pH1.0} - (A_{510\text{nm}} - A_{700\text{nm}})_{pH4.5} \quad (2)$$

M_W is the molecular weight of the main anthocyanin (Cyanidin-3-glycoside = 449.2 g/mol); D_f is the dilution factor (here is 10); 1000 is the mass conversion factor (g to mg); ε is the molar absorptivity of C3G (26.900 L/moL cm); and l is the path length (1 cm).

Total polyphenol content

The total polyphenol (TP) content in extracts was determined by the Folin Ciocalteu colorimetric method according to Azghandi Fardaghi et al. (2021). The Folin–Ciocalteau method measures reduced activity in samples. An aliquot (250 ml) of diluted sample extracts and gallic acid standards were placed in test tubes. Then 1.25 mL of Folin–Ciocalteus phenolic reagent (diluted in distilled water at a ratio of 1:10) was sequentially added to each tube. The solution was vortexed thoroughly and then incubated at room temperature (23 °C) for 5 min. Then it was made alkaline with 1 mL Na_2CO_3 (75 g/l). The test tubes were then placed in a dark place for 60 min, and the absorbance at 760 nm was recorded against the reagent blank. The concentration of total polyphenols was calculated using the calibration curve (100–500 mg/L) of gallic acid. Results were expressed as mg gallic acid equivalents per 100 g sample dry weight. Each experiment was repeated thrice, and the mean values were reported [34].

Antioxidant activity

DPPH radical-scavenging activity

After plotting the standard curve of different concentrations of 2,2-diphenyl–1-picrylhydrazyl, 1 mL of the extract in methanol (100–1000 µL/mL) was mixed with 2 mL of DPPH solution (0.004%, w/v in MeOH) and samples were stored in darkness place at a temperature of 25 °C for 30 min. Then, the absorbance of samples at 517 nm was measured by UV–Vis spectrophotometer (DR 5000™, Hach Canada). Antioxidant activity was assessed using the DPPH free radical method by Arruda et al. (2017) [35]. A similar procedure was carried out for blank; methanol was used instead of the extract. The DPPH radical scavenging activity was obtained according to the following Eq. (3):

$$\text{DPPH Scavenging activity (\%)} = \frac{A0 - A1}{A0} \times 100 \quad (3)$$

where A_0 and A_1 are the blank and sample solution absorbance, respectively. The sample concentration providing 50% inhibition (IC_{50}) was calculated by exponential regression analysis in Microsoft Excel 2021 (Microsoft, Inc.).

Ferric reducing antioxidant power assay

The antioxidant capacity-iron reduction test was based on the method of Hassanpour et al. [36]. The FRAP reagent was prepared from 2.5 mL of 10 mM TPTZ solution, 2.5 mL of 20 mM ferric chloride solution, and 25 mL of 300 mM acetate buffer (pH 3.6) and kept at 37 °C for 30 min. A 3 mL aliquot of the freshly prepared FRAP solution and 150 µL of the mixed extract were mixed and left in the dark place for 30 min before reading the absorbance at a wavelength of 593 nm. An aqueous solution of iron(II) sulfate ($FeSO_4.7H_2O.$) was used to create a calibration curve [36, 37].

Pectinase activity determination

The pectinase activity was measured according to the following method. Briefly, 50 µL of free pectinase solution (0.025 mg protein/mL in acetate buffer, pH 5.0) was added to 250 µL of pectin solution (1 mg/mL, acetate buffer, pH 5.0) at 30 °C. The mixture was gently shaken for a reaction time of 30 min, then 300 µL of 3,5-dinitro salicylic acid (3,5-DNA reagent) consisting of 3,5-DNA (1%, w/v), potassium sodium tartrate (30%, w/v) and sodium hydroxide (1%, w/v) was added into the reaction mixture. The samples were kept in boiling water for 10 min and then cooled in an ice-water bath. Samples were diluted with water (4.4 mL), and absorbance values were measured at 540 nm [38]. For this purpose, 40 mL of enzymatic solution (Polygalacturonase) at a specific concentration was added to 20 g *Berberis vulgaris* L. fruit, and the extraction was carried out. Sodium acetate buffer (50 mM) was used as a solvent to maintain optimal pH conditions. The enzymatic extraction was accomplished in a thermostatically controlled orbital shaker (IKA, KS 4000 I control, Germany) with gentle agitation (150 rpm) in the dark place. At the end of each extraction, the sample was centrifuged at 4 °C for 10 min at 2500 × g. Finally, the supernatant was filtered using Whatman No. 41 filter paper and kept in a brown flask at −20 °C until further analysis [32].

Statistical analysis

The analysis of variance (ANOVA) was performed, and the fitness of the model equation was evaluated using lack of fit (model error, coefficient of determination (R^2), adjusted-R^2(adj R^2), coefficient of variation (CV), and the Fisher test value (*F*-value). The statistical significance of the model and model variables were determined based on an *F* test at a probability level (*p*) of 0.01 or 0.05. The effect of variables was displayed in three-dimensional response surfaces contour plots. The barberry fruit properties were evaluated in triplicate. Design Expert (Version 13, Minneapolis, USA) was used for response optimization (RSM) of the extraction conditions.

Result and discussion

Chemical properties of fresh *Berberis vulgaris* L. fruit

The results of *Berberis vulgaris* L. chemical properties, including the total polyphenol, the amount of anthocyanin, and antioxidant activity, are reported in Table 1. According to the findings, the total phenolic and anthocyanin content of *Berberis vulgaris* L. fruit was 416.51 ± 10.27 mg/L and 223.97 ± 19.36 mg/L, respectively. Its antioxidant activity based on DPPH (IC50) and Ferric reducing antioxidant power methods was 16.78 ± 0.21 mg/mL and 2341.2 ± 0.02 µM, respectively. The fruit had 1.01 ± 0.02 g/100 g ash and 1.00 ± 0.01 g/100 g protein, 10.14 g/100 g fiber, 80.65 ± 0.23 g/100 g moisture and 12.14 ± 0.04 g/100 g reducing sugar. Naji-Tabasi et al. (2021) also reported *that Berberis vulgaris* L. fruit had total phenolic content of 221.45 (mg gallic acid/100 g). The antioxidant activity level in barberry fruit and fruit pulp were 80.53 and 67.54%, respectively. In addition to polyphenols content, high fiber exists in *Berberis vulgaris* L. pulp (32%). They noticed that the protein value in the *Berberis vulgaris* L. fruit is negligible [39].

Table 1 Chemical properties of *Berberis vulgaris* L

Chemical properties of *Berberis vulgaris* L	Unit	Average ± SD
Ash	g/100 g	1.01 ± 0.02
Protein	g/100 g	1.00 ± 0.01
Fiber	g/100 g	10.14 ± 0.27
Moisture	g/100 g	80.65 ± 0. 23
Reducing sugar	g/100 g	12.14 ± 0.04
DPPH (IC50)	mg/mL	16.78 ± 0.21
Ferric reducing antioxidant power assay	µmol/L	2341.2 ± 0.02
Anthocyanin content	mg/L	223.97 ± 19.36
Polyphenols content	mg/L	416.51 ± 10.27

Model fitting and optimization of pulsed electric field pretreatment

A quadratic regression model was the best model for response variables. For each response variable, the experimental data were fitted to the models. As presented in Tables 2 and 3, the regression models were highly significant, with satisfactory coefficients of determination ($R^2 > 0.97$). The R^2 values were close to one, indicating a high correlation between the observed and predicted values. These values also give an excellent fit to the mathematical model. The adjusted determination coefficients (adj $R^2 > 0.95$) showed the model adequacy and confirmed that the models were highly significant. The CV values lower than 5% for all responses represented excellent precision and reliability of the results. The lack-of-fit test, which measures the model's competence, did not result in a significant F value and showed that the models were adequate for predicting responses in the anthocyanin extracts within the ranges of variables used.

The analysis of variance showed that pulsed electric fields significantly affect the amount of anthocyanin of *Berberis vulgaris* L. extract under different electric currents and pulses ($p < 0.05$). ANOVA tables for the quadratic model for predicting total anthocyanins and polyphenols content

Table 2 ANOVA for response surface quadratic model for anthocyanins content

ANOVA for response surface quadratic model for anthocyanins content						
Source	**Some of Squares**	**df**	**Mean Square**	***F* Value**	***p*-value Prob > *F***	
Model	1694.66	5	338.93	7.17	0.0112	Significant
A-pulse	67.13	1	67.13	1.42	0.2723	
B-voltage	885.73	1	885.73	18.73	0.0034	
AB	236.39	1	236.39	5.00	0.0604	
A^2	186.77	1	186.77	3.95	0.0872	
B^2	127.65	1	127.65	2.70	0.1444	
Residual	330.99	7	47.28			
Lack of fit	252.59	3	84.20	4.30	0.0965	Not significant
Pure error	78.41	4	19.60			
Cor total	2025.65	12				

Table 3 ANOVA for response surface quadratic model for polyphenols content

Analysis of variance table for polyphenols content						
Source	**Some of Squares**	**df**	**Mean Square**	***F* Value**	***p*-value Prob > F**	
Model	2235.68	5	447.14	47.54	< 0.0001	Significant
A-pulse	1538.56	1	1538.56	163.59	< 0.0001	
B-voltage	482.41	1	482.41	51.29	0.0002	
AB	101.61	1	101.61	10.80	0.0134	
A^2	111.08	1	111.08	11.81	0.0109	
B^2	28.43	1	28.43	3.02	0.1257	
Residual	65.83	7	9.40			
Lack of fit	47.28	3	15.76	3.40	0.1341	Not significant
Pure error	18.56	4	4.64			
Cor total	2301.51	12				

(Tables 2 and 3) showed a high *F* value (18.73), indicating that voltage significantly changed the anthocyanins content. The pulse changes the total phenolic content according to a high *F* value (163.59). The quadratic model was chosen for anthocyanins and polyphenols contents according to the significance of the *F* test, the non-significance of the lack of fit value, and the R^2 and coefficient of variation values.

The optimization of *Berberis vulgaris* L. anthocyanins extraction with pulsed electric pretreatment was done to obtain the maximum extraction efficiency. The optimum conditions for the pulse electric field were 7000 V/cm voltage and pulse numbers of 100, which anthocyanin and polyphenol were 260.28 mg/L and 462.75 mg/L, respectively. The proposed treatment was produced under the same conditions as other treatments to verify the optimized extraction process. The experimental values for total anthocyanins and polyphenols content at optimum extraction were 260.28 mg/L and 462.75 mg/L, respectively. There was no considerable difference between the models ($P < 0.05$) and the experimental results, which proved the models' accuracy. Equations 4 and 5 showed the quadratic models for predicting total anthocyanins and polyphenols content.

$$\begin{aligned}\text{Anthocyanin} = &+ 361.83116 - 2.60854\text{Pulse} \\ &+ 0.022452\text{voltage} + 0.000154\text{Pulse} \\ &* \text{voltage} + 0.013157\text{Pulse}^2 \\ &- 1.69957E - 06\text{voltage}^2\end{aligned} \tag{4}$$

$$\begin{aligned}\text{Polyphenol} = &+ 434.70092 - 1.38548\text{Pulse} \\ &+ 0.004944\text{voltage} + 0.000101\text{Pulse} \\ &* \text{voltage} + 0.010147\text{Pulse}^2 \\ &- 8.02069E - 07\text{voltage}^2\end{aligned} \tag{5}$$

Table 4 Effects of PEF extraction conditions on (TP) and (TA) of *Berberis vulgaris* L. fruits

Run	Factor values		Response values	
	Number of pulses	Electric current (A)	Anthocyanins content (mg/L)	Polyphenols content (mg/L)
1	100	3000	223.38	432.51
2	50	3000	240.78	413.78
3	75	5000	233.60	428.91
4	75	7000	250.34	434.35
5	50	5000	224.78	417.93
6	75	3000	220.49	420.87
7	50	7000	246.93	423.86
8	100	7000	260.28	462.75
9	75	5000	230.54	430.88
10	75	5000	227.70	428.13
11	100	5000	248.90	456.39
12	75	5000	225.30	433.55
13	75	5000	222.23	429.23

Investigating the trend of changes in the content of total anthocyanin and phenolic compounds

Table 4. shows the effects of PEF extraction conditions on (TP) and (TA) of *Berberis vulgaris* L. fruits. In this regard, Fig. 1 presents the surface plot of TPC as a function of the electric field and the number of pulses. An increment in both variables can be observed as the electric field increases up to 7000 V/cm. This behavior might be related to an increment in the release of bioactive compounds and provide antioxidant activity, as Martínez et al. [40] observed in their study on berries [40]. Based on the data obtained in Fig. 1, the total phenol content of extract increased with the increment of number of pulses and the electric current of the pulsed electric field. Due to the complexity of chemical reactions in natural systems, explaining the increase in phenolic content is the whole problem. The results of this section were consistent with the results of Tena et al. [41]. The electroporation is the reason of this phenomenon, which a strong electric field permeabilizes the cell membrane, allowing ions and macromolecules to exit the cell [42]. Donsì et al. [43] illustrated that a 20 kJ/kg PEF treatment applied to grape skins after soaking in red wine allowed a 25% increase in antioxidant activity. Bobinaitė et al. [44] applied PEF to juice production and extraction of bioactive compounds from blueberries and observed an antioxidant activity increase of 36% when the energy used is 10 kJ/kg [44]. Pataro et al. [45] applied a 10 kJ/kg treatment to sweet cherries and achieved a 27% increase in antioxidant activity [45].

PEF treatment led to depolymerization of tannins, as revealed by analysis of polyphenols content. This may contribute to a higher release of antioxidant bioactive compounds, as Delsart et al. [46] observed in Cabernet Sauvignon grapes [46]. PEF treatment depolymerizes skin

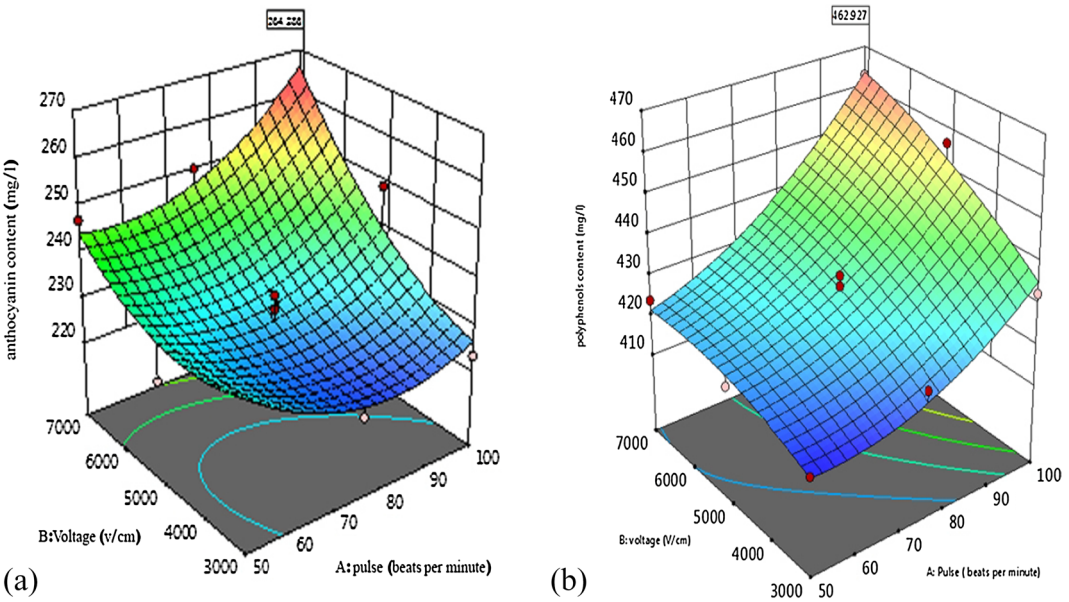


Fig. 1 Surface plots for pulsed electric field pretreatment variables on total anthocyanin content (TAC) (**a**) and polyphenols content (TPC) (**b**)

Table 5 Determination of total anthocyanins and polyphenols content from cold plasma pretreatment

Run	Factor values		Response values	
	Power (w)	Time (min)	Anthocyanins content (mg/L)	Polyphenols content (mg/L)
1	70	3	228.25	420.86
2	80	1	240.76	423.64
3	60	5	226.78	420.14
4	80	5	256.32	433.71
5	70	5	238.21	422.13
6	70	3	226.86	421.95
7	70	3	225.37	419.7
8	70	3	230.45	418.13
9	60	1	220.14	419.72
10	70	1	221.39	420.36
11	60	3	224.62	420.98
12	70	3	231.14	417.59
13	80	3	235.14	418.63

Table 6 ANOVA for response surface quadratic model for anthocyanins content

Analysis of variance table for anthocyanins content						
Source	Some of Squares	df	Mean Square	*F* Value	*p*-value Prob > F	
Model	1010.24	5	202.05	11.76	0.0027	Significant
A-Power	613.68	1	613.68	35.71	0.0006	
B-time	253.76	1	253.76	14.76	0.0064	
AB	19.89	1	19.89	1.16	0.3177	
A^2	38.87	1	38.87	2.26	0.1763	
B^2	37.23	1	37.23	2.17	0.1846	
Residual	120.31	7	17.19			
Lack of fit	97.03	3	32.34	5.56	0.0655	Not significant
Pure error	23.28	4	5.82			

tannins, resulting in smaller diffusing molecules. Increasing the electric field above 1300, V/cm reduces AA and TPC, depleting bioactive compounds. High electrical energy during PEF treatment leads to the degradation of valuable compounds [47]. Bobinaitė et al. [44] observed reduced TPC and AA on blueberry juice with increasing electric field up to 5 kV/cm after PEF pretreatment [44].

Model fitting and optimization of vacuum-cold plasma pretreatment

The best model for the response variable was the quadratic model. As shown in Tables 5 and 6, models were significant, with a satisfactory coefficient of determination ($R^2 > 0.95$) that indicated a good correlation between observed and predicted values. The adjusted determination coefficients (adj $R^2 > 0.94$) showed the model's fit and confirmed that the model was significant. CV values for all responses were below 5%, indicating the results' accuracy and reliability. The lack-of-fit test, which measures the model's competence, did not result in a significant *F* value and showed model adequacy. The optimum conditions for vacuum-cold plasma were obtained at the power of 80 w and time of 5 min, and the amount of anthocyanin and polyphenol was 256.32 and 433.71 mg/L, respectively.

The proposed treatment was produced under the same conditions as other treatments to verify the optimized extraction process. Predicted and experimental values of the response at optimum extraction for total anthocyanins content were 254.03 and 256.32 mg/L, respectively, and for polyphenols content were 432.97 and 433.71 mg/L, respectively. There was no significant difference between the models ($P < 0.05$) and the experimental efficiency observations, proving the models' accuracy. Moreover, Eqs. (6) and (7) showed the quadratic models for predicting total anthocyanins and polyphenols content.

Table 7 ANOVA for response surface quadratic model polyphenols content

Source	Sum of squares	df	Mean square	*F*-value	*p*-value	
Model	238.75	5	47.75	13.67	0.0017	Significant
A-Power	38.20	1	38.20	10.94	0.0130	
B-time	90.17	1	90.17	25.81	0.0014	
AB	61.23	1	61.23	17.53	0.0041	
A^2	36.62	1	36.62	10.48	0.0143	
B^2	0.9335	1	0.9335	0.2672	0.6211	
Residual	24.45	7	3.49			
Lack of fit	2.63	3	0.8782	0.1610	0.9173	Not significant
Pure error	21.82	4	5.45			
Cor total	263.20	12				

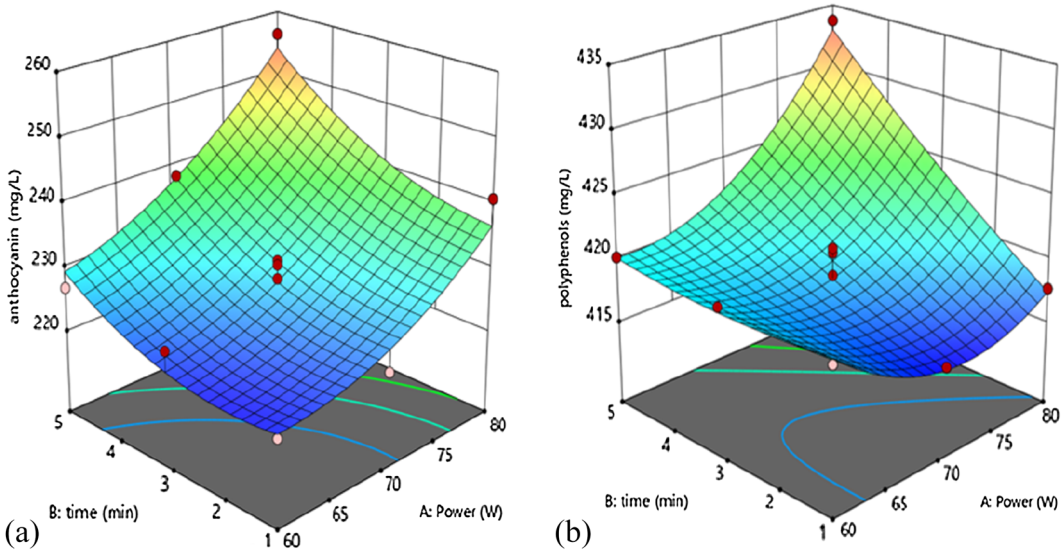


Fig. 2 Surface plots for cold plasma pretreatment variables on total anthocyanin content (TAC) (**a**) and polyphenols content (TPC) (**b**)

$$\begin{aligned}\text{Anthocyanin} = {} & +362.70589 - 4.57510\text{Power} \\ & - 10.06040\text{time} + 0.111500\text{Power} \\ & * \text{time} + 0.037514\text{Power}^2 \\ & + 0.917845\text{time}^2\end{aligned} \tag{6}$$

$$\begin{aligned}\text{Polyphenols} = {} & +615.77964 - 5.43247\text{Power} \\ & - 12.62749\text{time} + 0.195625\text{Power} * \text{time} \\ & + 0.036414\text{Power}^2 + 0.145345\text{time}^2\end{aligned} \tag{7}$$

Analysis of the variance table for anthocyanins content is shown in Table 6. The F value (35.71) indicated that power significantly changes anthocyanin content. ANOVA table of the quadratic model for total polyphenols content was also shown in Table 7, in which the F value (25.81) announced that time causes a fundamental change in total phenolic content. The results of this section agreed with the results of Zhou et al. (2022) [48].

The effects of vacuum-cold plasma extraction conditions on TPC and TA are provided in Table 5. Figure 2(b) illustrates the surface plot of TPC. It was observed that the total phenol content of the extract increased with increasing power and time of the cold plasma pretreatment. The results of this section were consistent with the results of Keshavarzi et al. [21]. They showed that total phenolic content and antioxidant activity of green tea increased by cold plasma at 15 W for 15 min (41.14% and 41.06%, respectively [21]. The main physical change observed after cold plasma pretreatment is increasing of surface roughness and topography after treatment. The increase in surface roughness is due to the plasma etching effect.

Etching is more related to bombarding the surface using high-energy plasma particles, such as electrons, ions, radicals, neutral particles, atoms, and molecules excited by the surface. Physical etching leads to the removal or re-accumulation of fragments with low molecular weight, and chemical etching leads to the breaking of chemical bonds, chain cutting, oxidation, or chemical destruction of treated materials [49, 50]. There are few studies on optimizing the extraction of bioactive components by cold plasma treatment in plant materials. Mehta et al. [50] studied the effect of cold atmosphere and vacuum plasma on the extraction yield of polyphenols from de-oiled rice and corn bran. Phenolic compounds from rice and corn bran were investigated for bioactivity, digestibility, cytotoxicity, and anti-inflammatory activities. Cold plasma treatment resulted in a significant increase ($P < 0.05$) [50].

Moreover, there was a significant increase in total phenolic content, total flavonoid content, and antioxidant activity. In line with our work, Kumar et al. (2023) have also stated that the impact of cold plasma on polyphenols content mainly depends on the food matrix and plasma process parameters, viz. voltage, feed gas, and treatment time. Among various polyphenols, flavonoids are degraded faster because of their high ability to scavenge plasma-generated free radicals. The reactive species cause oxidative degradation, double bond cleavage of polyphenol compounds, and aid in extracting phenolic compounds. Cold plasma technology positively and negatively impacts polyphenol concentration [51].

Model optimization and verification for enzymatic pretreatment

Linear models were chosen according to the significance of the F test, the non-significance of the lack of fit value, and the R^2 and coefficient of variation values. The optimum conditions for enzymatic pretreatment were obtained at the enzyme concentration of 1.5% and temperature of 60 °C. In the optimum condition, anthocyanin and polyphenol were 279.64 and 484.93 mg/L, respectively. Experimental values at optimum extraction for total anthocyanins and polyphenols content were 279.64 and 484.71 mg/L, respectively. There was no significant difference between the predicted and experimental efficiency observations ($P < 0.05$), which approved the models' accuracy. Equations (8) and (9) showed the quadratic models for predicting total anthocyanins and polyphenols content.

$$\text{anthocyanin} = +\ 233.54051 + 13.02667\text{consentration} + 0.447333\text{Temperature} \quad (8)$$

$$\text{Polyphenols} = +\ 438.31487 + 13.80000\text{consentration} + 0.428333\text{Temperature} \quad (9)$$

Table 8 ANOVA for Response Surface linear model for anthocyanins content

ANOVA for Response Surface Linear model for anthocyanins content						
Source	**Some of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	374.61	2	187.30	44.68	< 0.0001	Significant
A-concentration	254.54	1	254.54	60.72	< 0.0001	
B-Temp	120.06	1	120.06	28.64	0.0003	
Residual	41.92	10	4.19			
Lack of fit	29.45	6	4.91	1.57	0.3439	Not significant
Pure error	12.47	4	3.12			

Table 9 ANOVA for Response Surface linear model polyphenols content

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	395.74	2	197.87	135.34	< 0.0001	Significant
A-concentration	285.66	1	285.66	195.38	< 0.0001	
B- Temperature	110.08	1	110.08	75.29	< 0.0001	
Residual	14.62	10	1.46			
Lack of fit	4.28	6	0.7133	0.2759	0.9217	Not significant
Pure error	10.34	4	2.59			
Cor total	410.36	12				

Table 10 Determination of total anthocyanins and polyphenols content from enzymatic method

Run	Factor values		Response values	
	Concentration (%)	Temp (°C)	Anthocyanins content)mg/L(	Polyphenols content)mg/L(
1	0.5	60	265.37	470.29
2	1.5	60	279.64	484.93
3	1	60	273.28	478.45
4	1	50	272.89	471.13
5	1	40	262.28	467.89
6	0.5	40	258.76	463.23
7	1	50	270.68	473.9
8	1.5	40	270.41	476.85
9	1	50	269.21	474.32
10	1	50	268.45	472.93
11	1	50	271.43	475.39
12	0.5	50	260.29	466.73
13	1.5	50	273.45	479.87

Determination of total anthocyanins and polyphenols contents from enzymatic pretreatment

ANOVA tables for linear models for anthocyanins and polyphenols contents are shown in Tables 8 and 9. The F values of total anthocyanins (60.72) and polyphenols contents (195.38) indicate that concentrations make an impressive change in the total anthocyanins total phenolic contents. Table 10 and Fig. 3 summarize the effects of enzymatic method extraction on TPC and TA. The results showed that the total phenols and anthocyanins contents increased with increasing pectinase enzyme concentration and treatment temperature. An increment in both variables can be observed when the concentration increases to 1.5%. The results of this section were consistent with the results of Kim et al. [52]. They reported pectinase-assisted extraction method was optimized to enhance the total phenolic (TP) content and total anthocyanin (TA) content from mulberry (*Morus alba* L.) fruit extracts. The optimal conditions were 1:5 w/v material/

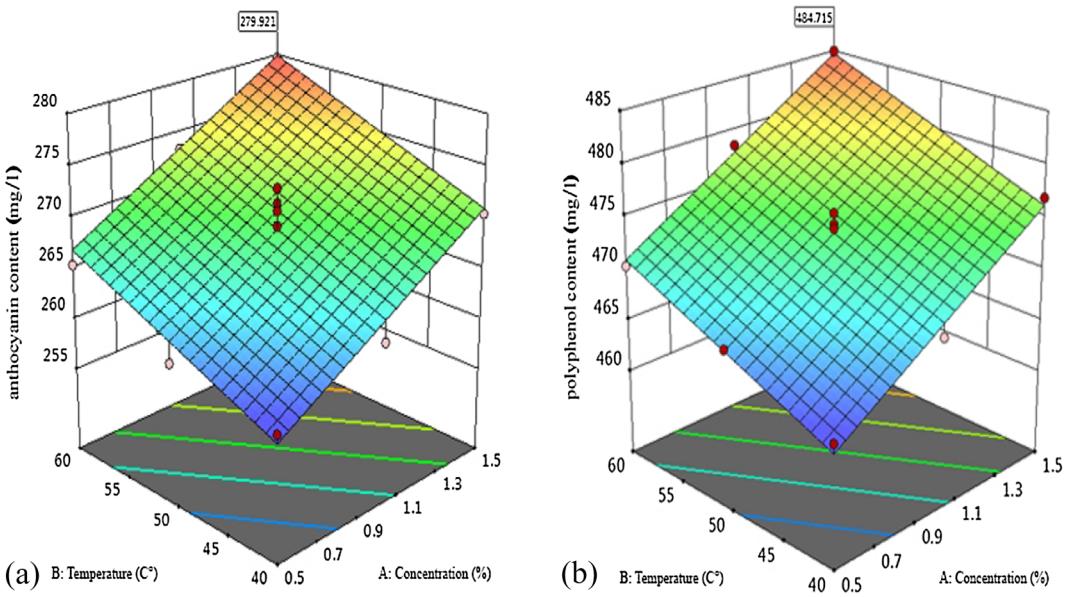


Fig. 3 Surface plots for Pectinase pretreatment variables on total anthocyanin content (TAC) (**a**) and polyphenols content (TPC) (**b**)

Table 11 Predicted and experimental values of the response variables at optimum extraction

Characteristics	pulsed electric field		Cold plasma		Enzymatic method	
	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental
TAC (mg/L)	261.950	260.28	254.03	256.32	279.92	279.64
TPC (mg/L)	462.417	462.75	432.97	433.71	484.71	484.93

water ratio, 3.5% pectinase (v/w), and 1.5% citric acid (w/w) with a 113 min reaction time at 50 °C. Under these conditions, total phenolic TPC and total anthocyanin (TA) content were significantly increased compared with the untreated control. González et al. (2022) surveyed a comparison study between ultrasound–assisted and enzyme–assisted extraction of anthocyanins from blackcurrant (*Ribes nigrum* L.); the results suggested that TPC and TA were significantly increased [53]. Enzymes disrupt the complex cell wall structure to release the active constituents [54].

Zhang et al. [55] investigated ultrasonic-assisted enzymatic extraction and identification of anthocyanin components from mulberry wine residues, and the optimized conditions increased anthocyanin yield through improved utilization of mulberry wine residues [55].

Comparing different methods of extraction at optimum condition

The efficacy of the optimum condition of vacuum-cold plasma, pulsed electric field, and enzymatic pretreatments were compared in Table 11. The enzymatic pretreatment makes an impressive change in the total anthocyanins [279.64 (mg/L)] and the total phenolic contents [484.93 (mg/L)] compared with vacuum-cold plasma (TAC: 256.32 mg/L) and TPC: 433.71 mg/L) and pulsed electric field (TAC: 260.28 mg/L) and (TPC: 462.75 mg/L). As a result, enzymatic pretreatment extraction had higher efficiency for extraction of anthocyanins and polyphenols and can be applied for extraction of nutraceuticals of barberry with high efficiency.

Conclusion

The main objective of this study was to investigate the impact of pulsed electric field, vacuum-cold plasma, and enzymatic pretreatments on the extraction efficiency of polyphenols and anthocyanins from *Berberis vulgaris* L. The optimum conditions for the electric pulse field were cold plasma pretreatment and for enzymatic pretreatment were 7000 (V/cm) with n = 100, 80 W for 5 min, and 1.5% enzyme at 60 °C. The maximum value of anthocyanins in the optimum condition of pulsed electric field, vacuum-cold plasma, and enzymatic pretreatment were 260.28 mg/L, 256.32 mg/L, and 279.64 mg/L, respectively. In optimum conditions, the maximum value of polyphenols extracted by pulsed electric field, vacuum-cold plasma, and enzymatic pretreatment were 462.75 mg/L, 433.71 mg/L, and 484.93 mg/L, respectively. In a comparative estimate with another conventional extraction method, all of these pretreatments significantly enhanced the content of polyphenols and anthocyanins amounts from *Berberis vulgaris* L. ($p < 0.05$). The comparison between different pretreatments showed that the enzymatic pretreatment resulted in the highest anthocyanins and polyphenols extraction efficiency. The enzymes disrupt the complex cell wall structure and release the active constituents more effectively.

Abbreviations

CCD	Central composite design
DSE	Direct solvent extraction
DPPH	1, 1-Diphenyl-2-picrylhydrazyl-hydrate
FRAP	Ferric reducing antioxidant power assay
TPTZ	2, 4, 6-Tris (2-pyridyl)-s-triazine
GAE	Gallic acid equivalents
TPC	Total phenolic content
TAC	Total anthocyanin content
C3G	Cyanidin-3-glycoside
PEF	Pulsed electric field

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Author contributions

AD: data collection, writing the manuscript draft, data analysis, and interpretation. JF: presenting the research idea and study design, revising and editing the manuscript, supervising the study, and approving the final version. SN-T: presenting the research idea and study design, revising and editing the manuscript, supervising the study, and approving the final version. EF: supervising the study, approval of the final version. AR: supervising the study, approval of the final version.

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Declarations

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Consent to Participate

Not applicable.

Competing interests

The authors declared that there is no competing interest.

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