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Optimization of Ultrasound-Assisted Extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium ashei*) wine pomace

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ABSTRACT

Ultrasound-Assisted Extraction (UAE) of total anthocyanins (TA) and phenolics (TP) from Blueberry Wine Pomace (BWP) was optimized using Response Surface Methodology (RSM). A Box–Behnken design was used to predict that the optimized conditions were an extraction temperature of 61.03 °C, a liquid–solid ratio of 21.70 mL/g and a sonication time of 23.67 min. Using the modeled optimized conditions, the predicted and experimental yields of TA and TP were within a 2% difference. The yields of TA and TP obtained through the optimized UAE method were higher than those using a Conventional Solvent Extraction (CSE) method. Seven anthocyanins, namely delphinidin-3-*O*-glucoside, delphindin-3-*O*-arabinoside, petunidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside and malvidin-3-*O*-arabinoside, were found in the BWP extract from both the UAE and CSE methods.

1. Introduction

Blueberry belongs to the genus *Vaccinium* and family Ericaceae and originates from North America and Europe (Rimando, Kalt, Magee, Dewey, & Balling, 2004). Blueberry is a popular fruit around the world, and in recent years is popular in China, where it has been cultivated since 1989. Like most berries, blueberries contain abundant phenolic compounds, such as anthocyanins, flavonols and chlorogenic acids (Seeram et al., 2006). The rabbiteye blueberry is one of the richest plant sources of anthocyanins (a class of flavonoids). This blueberry contains several distinct anthocyanins, such as cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, malvidin-3-*O*-galactoside, and peonidin-3-*O*-galactoside (Muller, Schantz, & Richling, 2012), that are dark red, blue or purple in color depending on the pH (Li et al., 2012). As a consequence of the social trend towards the consumption of natural instead of synthetic food components, anthocyanins have received increasing attention for use as natural colorants in the food industry (Verbeyst, Crombruggen, Plancken, Hendrickx, & Loey, 2011). In addition, anthocyanins contribute to the beneficial health effects of blueberry, providing protection against several chronic diseases, including cardiovascular disorders (Gaziano et al., 1995), neurodegenerative diseases (Prior & Wu, 2006) and cancer (Routray & Orsat, 2010).

Blueberries are suitable for processing into wine or juice because of their acidity, sugar and anthocyanin content. Anthocyanins are abundantly present in the skin of blueberries (Kim, Bartley, Rimando, & Yokoyama, 2010), therefore, the pomace formed in the wine or juice-making process contains many phenolic and other bioactive compounds (Melo et al., 2015; Su & Silva, 2006). The pomace is a rich source of these and other health-promoting compounds, which can be retrieved through extraction (Gonzalez-Centeno et al., 2014).

Recently, improved methods have been developed to extract bioactive compounds from plants, for example, the use of enzymes (Puri, Sharma, & Barrow, 2012), Ultrasound-Assisted Extraction (UAE) (Ramic et al., 2015; Teng, Lee, & Choi, 2014), microwaves (Valdes, Vidal, Beltran, Canals, & Garrigos, 2015) and supercritical fluid (Meneses, Caputo, Scognamiglio, Reverchon, & Adami, 2015). Compared with Conventional Solvent Extraction (CSE) methods, the use of ultrasound for extraction of phenolic compounds has been reported as a faster, more highly efficient, and solvent-saving technique (Tao, Wu, Zhang, & Sun, 2014). Ultrasonic

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waves generate a cavitation effect in the solvent, resulting in faster movement of molecules and higher penetration of the solvent into the target material (Toma, Vinatoru, Paniwnyk, & Mason, 2001). This results in an accelerated release of the target compounds (Avhad & Rathod, 2015). Hence, UAE is being widely used in the extraction of bioactive compounds from natural sources for use in food systems (Corbin et al., 2015). Furthermore, use of ultrasound reduces the use of toxic solvents, which is desirable for extracting bioactive compounds for human consumption.

Response Surface Methodology (RSM) is a mathematical and statistical tool that has been widely used to investigate the possible interactions between experimental variables in various processes (Almeida, Erthal, Padua, Silveira, & Amelia, 2008). In the present study, UAE parameters such as extraction temperature, sonication time and liquid–solid ratio were optimized using RSM in order to determine the conditions that would maximize the yields of TP and TA from Blueberry Wine Pomace (BWP) without running endless expensive trials. The optimized UAE protocol was used to extract TP and TA, and the yields from the UAE protocol were compared with those from CSE. The anthocyanins extracted from BWP by the UAE and CSE methods were identified by UPLC–DAD–MS/MS.

2. Materials and methods

2.1. Blueberry Wine Pomace (BWP) samples

BWP as by-product of blueberry winery, which was collected from brewing workshop of the Anhui Hui-king Food Company Limited (Hefei, China). The BWP was dried in an air-circulating oven at 30 °C for about 48 h, after which it was milled into powdered particles smaller than 0.6 mm (No. 30 mesh). The resulting powder was sealed and stored at 4 °C.

2.2. Chemicals and reagents

Gallic acid was obtained from Alfa Aesar Chemical Co., Ltd (China). Ethanol, sodium acetate, potassium chloride, formic acid and Folin–Ciocalteu reagent were of analytical grade and were purchased from Sigma–Aldrich Co., Ltd (USA). HPLC grade acetonitrile was purchased from Tedia Company, Inc. (USA).

2.3. Ultrasonic-Assisted Extraction (UAE)

The ultrasound equipment (Hong Xiang Long Biotechnology Co., Ltd; Beijing, China) was mainly composed of four parts: ultrasound generator, transducer, ultrasound cylinder probe, and beaker. The sample (BWP), extraction solvent of ethanol (70%, v/v) and hydrochloric acid (0.01%, v/v) were put in a 500 mL beaker. Acoustic cavitations were produced in the solvent by ultrasonic wave, and cavitations were observed from creation, growth and implosion of gas bubbles under the ultrasonic treatment. Ultrasonic power (400 w), extraction temperature and sonication time were controlled via the equipment panel.

2.4. Conventional Solvent Extraction (CSE)

CSE was carried out as a control group for comparison with UAE. Samples were extracted at 61 °C for 35 min, using ethanol (70%, v/v) and hydrochloric acid (0.01%, v/v) as solvents. The generated solids were separated from the mixture by centrifugation.

2.5. Screening of variables and experimental design

Numerous variables may affect the study, it is impractical to identify all those making small contributions. Therefore, those variables with major effects were sought and varied (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). Selection of the most suitable solvent was the initial step. Many reports suggested that ethanol was the most appropriate solvent for the extraction of various phenolic compounds from different plant materials (Chen, Zhao, & Yu, 2015; Gonzalez-Centeno et al., 2014). Additionally, ethanol is categorized as GRAS (Generally Recognized as Safe) for application in food systems (REF). Therefore, ethanol was chosen as the extraction solvent for these experiments.

Selection of UAE conditions were based on single factor experiments (Fig. S1, S2 and S3, Table 1). Influence of extraction temperature was investigated in the range from 50 to 70 °C, of liquid–solid ratio in the range of 15–25 mL/g, and of sonication time in the range from 15 to 35 min.

To investigate the relationship between the extraction conditions (extraction temperature, sonication time and liquid–solid ratio) and the yields of TA and TP, a three-level, three-factor Box–Behnken design (BBD) was employed to determine the combined variable conditions. The three independent variables were coded at three levels (−1, 0, +1) (Table 1). The response variables were fitted to the following second order polynomial model equation (Eq. (1)) which was able to describe relationship between the responses and the independent variables.

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

where, Y was the response variables (TA, TP); X_i and X_j were independent variables. β_0 was the constant coefficient; β_i was the linear coefficient; β_{ii} was the quadratic coefficient and β_{ij} was the cross-product coefficients.

2.6. Analysis of total anthocyanins (TA)

TA in the samples was estimated using the pH-differential method (Kunradi, Cristiane, Valdemiro, & Roseane, 2011) using two buffers, namely potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). Briefly, the samples were mixed with the pH buffer solutions and the absorbance was measured at 520 nm and 700 nm.

The concentration of anthocyanins in the extract was calculated and expressed as cyanidin-3-*O*-glucoside (C3G) equivalent according to Eq. (2).

$$\text{TA (mg/g)} = (A \times M_w \times \text{DF} \times V \times 1000)/M_a \times L \times m \quad (2)$$

where $A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$, M_w = molecular weight (449.2 g/mol), DF = dilution factor, M_a = extinction coefficient (26,900 mol/L * cm), L = path length (1 cm), m = the quantity of BWP (g), V = the total volume (mL), 1000 is convert from g to

Table 1
Experimental values and coded levels of the independent variables used for the Box–Behnken design.

Factor levels	Independent variable		
	Extraction temperature (°C)	Liquid-solid ratio (mL/g)	Sonication time (min)
−1	50	15	15
0	60	20	25
+1	70	25	35

mg. Results were expressed as mg of cyanidin-3-*O*-glucoside (C3G) equivalents per g of BWP (mg C3G/g_{BWP}).

2.7. Analysis of total phenolics (TP)

TP was determined using the Folin–Ciocalteu method (Flores, Singh, Kerr, Pegg, & Kong, 2014). Results were expressed as mg of gallic acid equivalent (GAE) per g of BWP (mg GAE/g_{BWP}).

2.8. Anthocyanins identification by UPLC–DAD–MS/MS

UPLC–DAD–MS/MS was used to identify compounds extracted from BWP by UAE and CSE. The chromatographic system consisted of an Acquity UPLC (Waters, Millford, US) equipped with a Diode Array Detector (DAD) and a Triple Quadrupole Mass Spectrometer. Samples were separated using an Agilent C_{18} column packed with Zorbax (2.1 × 50 mm, 1.7 μm; Agilent Technologies Co., Ltd., Shanghai, China). The column temperature was maintained at 30 °C. Aqueous formic acid (0.05%, v/v) and acetonitrile were used as mobile phase A and mobile phase B, respectively. The gradient program was as follows: 2–11% B (0–2 min), 11–16% B (2–8 min), 16–2% B (8–9 min), and 2% B (9–14 min). Equilibrium time between runs was 10 min. Anthocyanins were detected at 520 nm. For MS detection, the capillary voltage was set at 2500 V. The source temperature was held at 140 °C, and the desolvation temperature at 350 °C. The desolvation gas flow rate was 350 L/h (nitrogen), and the cone gas flow rate was 25 L/h (nitrogen).

2.9. Statistical analysis

All experiments were carried out in triplicates. The experimental results were expressed as means ± standard deviation ($n = 3$). Analysis of variance (ANOVA) was carried out to determine any significant differences ($P < 0.05$). Response surface plots were generated using Statistica 8.0 (Statsoft Inc., NY, USA)

Table 2
Box–Behnken design (BBD) experimental design with the independent variables and experimental data for the responses.

Run	Extraction conditions			Experimental results	
	Extraction temperature (°C)	Liquid–solid ratio (mg/L)	Sonication time (min)	TA (mg/g_{BWP})	TP (mg/g_{BWP})
1	60	20	25	4.08 ± 0.02	14.57 ± 0.08
2	60	15	15	3.68 ± 0.05	13.08 ± 0.03
3	70	15	25	3.41 ± 0.02	10.70 ± 0.03
4	50	20	15	4.02 ± 0.01	7.88 ± 0.02
5	60	15	35	3.65 ± 0.03	7.19 ± 0.11
6	50	25	25	3.93 ± 0.06	12.34 ± 0.04
7	60	20	25	4.09 ± 0.09	15.21 ± 0.01
8	60	20	25	4.11 ± 0.05	15.43 ± 0.08
9	60	25	15	4.02 ± 0.03	11.22 ± 0.06
10	70	20	35	3.82 ± 0.01	8.86 ± 0.10
11	60	25	35	3.73 ± 0.02	14.39 ± 0.08
12	50	20	35	3.96 ± 0.04	11.27 ± 0.07
13	70	20	15	3.92 ± 0.09	14.11 ± 0.02
14	60	20	25	4.11 ± 0.01	16.01 ± 0.03
15	70	25	25	3.83 ± 0.05	15.57 ± 0.09
16	50	15	25	3.84 ± 0.04	9.82 ± 0.05
17	60	20	25	4.10 ± 0.02	15.50 ± 0.12

Table 3
ANOVA for response surface polynomial model of all independent variables.

Factor	TA					TP				
	SS	DF	MS	*F*-value	*p*-Value	SS	DF	MS	*F*-value	*p*-Value
Model	0.6700	9	0.0740	21.61	0.0003**	132.10	9	14.68	59.72	<0.0001**
Linear term										
X_1	0.0740	1	0.0740	21.58	0.0024**	7.86	1	7.86	31.98	0.0008**
X_2	0.1100	1	0.1100	31.47	0.0008**	20.26	1	20.26	82.41	<0.0001**
X_3	0.0290	1	0.0290	8.38	0.0231*	2.62	1	2.62	10.67	0.0137*
Quadratic										
X_1^2	0.0480	1	0.0480	13.97	0.0073**	18.36	1	18.36	74.70	<0.0001**
X_2^2	0.3000	1	0.3000	87.22	<0.0001**	5.55	1	5.55	22.59	0.0021**
X_3^2	0.034	1	0.0340	9.76	0.0167**	31.28	1	31.28	127.27	<0.0001**
Interactions										
X_1X_2	0.0270	1	0.0270	7.93	0.0259*	1.38	1	1.38	5.62	0.0496*
X_1X_3	0.0004	1	0.0004	0.12	0.7429ns	18.66	1	18.66	75.93	<0.0001**
X_2X_3	0.0170	1	0.0170	4.92	0.0621ns	20.52	1	20.52	83.49	<0.0001**
Lack of fit	0.0150	3	0.0049	2.11	0.2421	0.63	3	0.21	0.77	0.5692
Pure error	0.0091	4	0.0023			1.09	4	0.27		
CV	2.16					3.95				
R^2	0.9655					0.9871				

X_1, X_2 and X_3 represent extraction temperature, liquid–solid ratio and sonication time, respectively; SS, DF, MS and CV represent sum of squares, degree of freedom, mean square, coefficient of variation, respectively.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
ns Not significant at $P > 0.05$.

3. Results and discussion

3.1. Modeling of the extraction process

Experimental modeling results for TA and TP were shown in Table 2. The experimental results of TA and TP in the BWP extracts varied from 3.41 to 4.11 mg C3G/g_{BWP} and 7.19 to 16.01 mg GAE/g_{BWP}, respectively. From model analysis, the R^2 value, F value and P value were determined for use in evaluating the mutual interaction of the independent and dependent variables (Table 3). A second order polynomial model (Eq. (1)) fitted the experimental data well, with R^2 values of 0.9655 and 0.9871 for TA and TP, respectively, which suggested the significance of the model at a 95% confidence level. For both responses, mathematical models were statistically acceptable due to significant regression ($P_m < 0.05$) and non-significant lack of fit ($P_{lf} > 0.05$). Furthermore, the coefficient of variance (CV), which represents the dispersion degree of the data, was rather low ($CV < 10\%$) in each model. This further supported the good fit of the model, and thus, provided better reproducibility. The second-order polynomial Eqs. (3) and (4) described the relationship between extraction temperature (X_1), liquid–solid ratio (X_2) and sonication time (X_3).

$$\begin{aligned}\mathrm{TA} = {} & -4.30031 + 0.087975X_1 + 0.38355X_2 + 0.070625X_3 \\ & + 0.00165X_1X_2 - 0.0001X_1X_3 - 0.0013X_2X_3 \\ & - 0.0010675X_1^2 - 0.01067X_2^2 - 0.0008925X_3^2 \end{aligned} \quad (3)$$

$$\begin{aligned}\mathrm{TP} = {} & -101.77219 + 2.91002X_1 + 0.31795X_2 + 1.65963X_3 \\ & + 0.011750X_1X_2 - 0.021600X_1X_3 + 0.045300X_2X_3 \\ & - 0.020882X_1^2 - 0.045930X_2^2 - 0.027258X_3^2 \end{aligned} \quad (4)$$

3.2. Effect of independent variables on TA and TP in the RSM model

From the regression evaluation, it could be observed that the three independent variables have a linear effect on the yields of TA and TP within the experimental UAE range. As shown in Table 3, the TA extraction yield was more significantly affected by extraction temperature and liquid–solid ratio at the level of $P < 0.01$, than by sonication time at the level of $P < 0.05$. The quadratic terms X_1^2 and X_2^2 were highly significant at $P < 0.01$, followed by X_3^2 at $P < 0.05$. The interaction of X_1X_3 and X_2X_3 were non-significant ($P > 0.05$). The TP extraction yield was more significantly affected

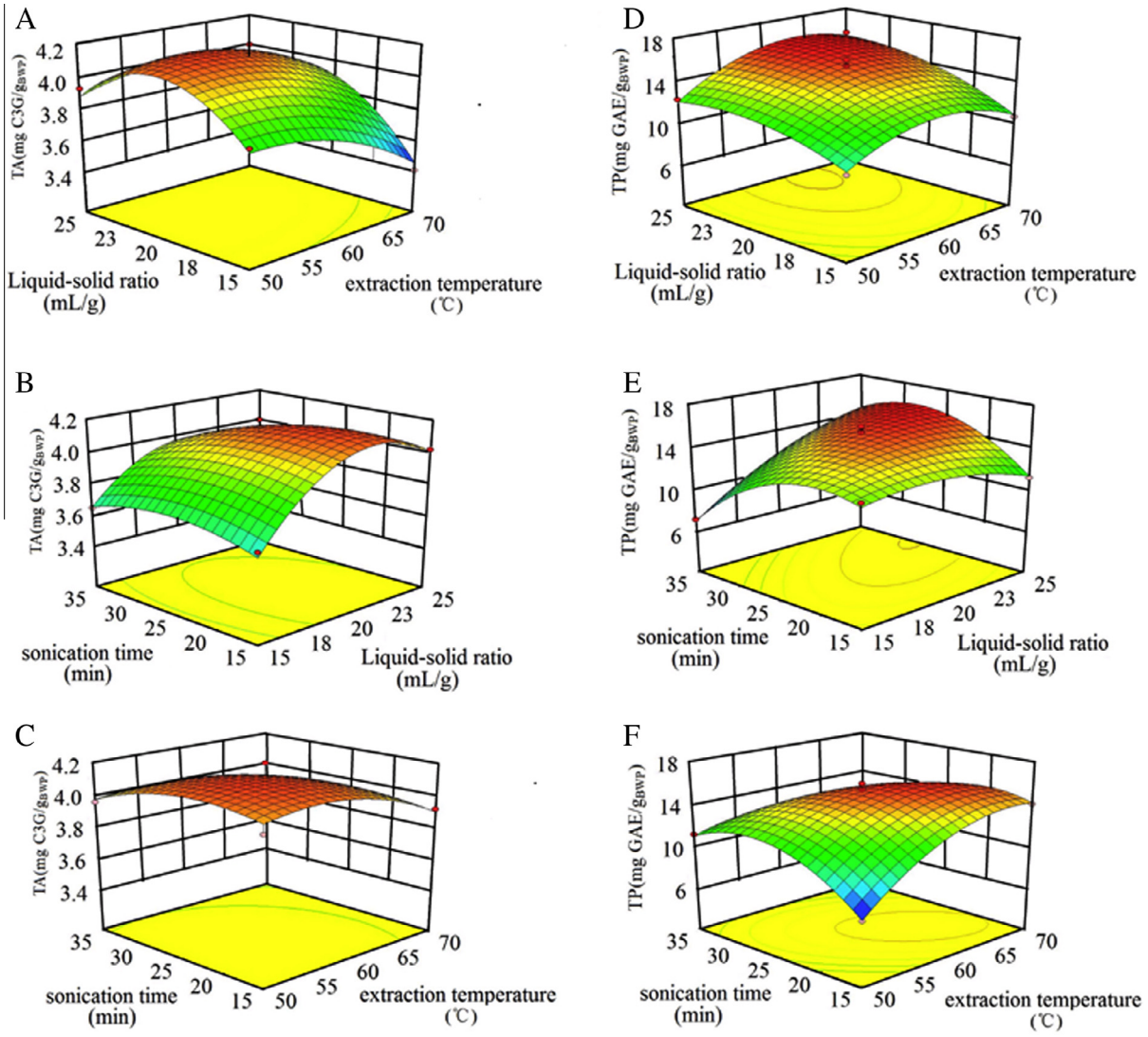

Fig. 1. Response surface plot for interactions between three independent variables (extraction parameters) on extraction yields of total anthocyanins (TA, in mg C3G/g_{BWP}, A, B, and C) and total phenolics (TP, in mg GAE/g_{BWP}, D, E, and F). Two variables were plotted against each other in each panel.

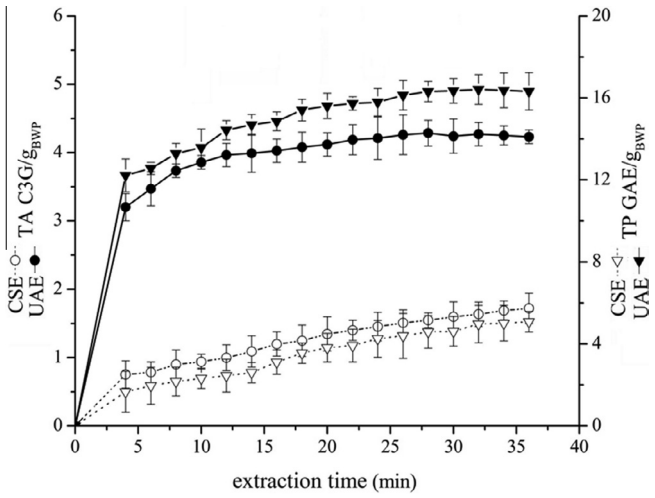

Fig. 2. Comparison of the total anthocyanins (TA, in mg C3G/g_{BWP}, circles) and total phenolics (TP, in mg GAE/g_{BWP}, triangles) extracted from BWP using UAE (filled) and CSE (open) at the optimized conditions (61 °C and 22:1 liquid–solid ratio).

by extraction temperature and liquid–solid ratio at $P < 0.01$, followed by sonication time at $P < 0.05$. All quadratic terms were more significant at $P < 0.01$. The interaction of X_1X_3 and X_2X_3 were statistically significant at $P < 0.01$, while X_1X_2 was significant at $P < 0.05$.

The experimental yield of TA increased as the three extraction factors were increased. Near the midpoint of the response plot, the yields reached their highest. The yield of TA was shown to be negatively influenced after extraction temperature and sonication time (Fig. 1A and C). Similarly, TA increased with the decrease in liquid–solid ratio (down to about 20 mL/g) at any fixed sonication time (Fig. 1B). Anthocyanins are highly sensitive to factors such as temperature, pH, oxygen, and water activity, often rendering their separation and purification expensive and time consuming (Alessandro, Kriaa, Nikov, & Dimitrov, 2012; Woodward, Kroon, Cassidy, & Kay, 2009). Extraction temperature was an important factor, with optimum temperature increasing the solubility of anthocyanins in the solvent (Alessandro et al., 2012), but temperatures too high leading to their degradation (Kechinski, Guimaraes, Norena, Tessaro, & Marczak, 2010; Ulrike, Reinhold, & Dietmar, 2013). In order to balance the extraction yields and loss of anthocyanins, an optimum temperature of 61 °C was chosen. Similarly, anthocyanins degrade rapidly in neutral and alkaline conditions, compared with acidic conditions (Woodward et al., 2009). In order to further improve the stability of anthocyanins, hydrochloric acid was used to adjust the pH of extraction solutions (pH = 2.0–2.3). The liquid–solid ratio was also a vital factor in increasing the TA yield, as an increased liquid–solid ratio could facilitate the access to the solute to the solvent. In addition, cavitation is known to produce a series of mechanical effects, such as particle collisions and cell wall disruption (Chemat, Huma, & Khan, 2011), which promote penetration of the solvent into the sample matrix and increase mass transfer rates of anthocyanins (Avhad & Rathod, 2015). The ultrasonic power might weak the cell wall (Ramic et al., 2015), increasing the contact between anthocyanins and solvent, which result in a reduction of the sonication time. However, some factors of sonication, such as power and frequency of ultrasonic waves, may cause adverse effects (Gonzalez-Centeno et al., 2014). Therefore, in order to gain maximum extraction yield, key parameters (sonication time, extraction temperature and liquid–solid ratio) were accurately determined.

The yield trends for TP (Fig. 1D, E, F) was similar to the trends of TA yield (Fig. 1A, B and C). The maximum experimental yield of TP was 16.01 mg GAE/g_{BWP} at the extraction temperature of 60 °C, liquid–solid ratio of 20 mL/g and sonication time of 25 min (Table 2). While a sufficiently, high temperature can increase the diffusion coefficient of the solute from the solvent and improve solute's solubility (Chen et al., 2015), a temperature higher than optimal can cause some thermo-sensitive compounds to degrade (Ghitescu et al., 2015; Vieira, Cavalcanti, Meireles, & Hubinger, 2013).

3.3. Verification of the predicted optimal extraction conditions

To verify the reliability of the models, an experiment was performed under the modified optimal conditions: 61 °C, 22:1 liquid–solid ratio and 24 min sonication time. The extraction yields for TA and TP were 4.19 mg C3G/g_{BWP} and 16.03 mg GAE/g_{BWP}, respectively, which were well matched with the predicted values of 4.12 mg C3G/g_{BWP} and 15.81 mg GAE/g_{BWP}. The errors between the predicted and experimental values were less than 2%. Thus, we extrapolated that the regression models obtained by RSM could predict the TA and TP extraction yields for any combination of liquid–solid ratio, extraction temperature and sonication time.

3.4. Comparison of extraction methods on yields of TA and TP

The effect of the extraction method (UAE or CSE at an extraction temperature of 61 °C and a 22:1 liquid–solid ratio) on the yield of the TA and TP were measured over time (Fig. 2). The UAE method resulted in a higher yields of both TP and TA than the CSE. The highest yields using UAE were reached at 30 min, resulting in 4.27 mg C3G/g_{BWP} TA and 16.41 mg GAE/g_{BWP} TP, while the highest yields using CSE were only 1.72 mg C3G/g_{BWP} and 5.08 mg GAE/g_{BWP} at 35 min using CSE. It was noteworthy that the UAE extraction yield of TA and TP were nearly 80% of the total at 4 min. Furthermore, the UAE method yielded about 2.5-fold higher TA and about 3.2-fold TP than the CSE method.

The production of higher TA and TP yields by UAE could be attributed to the ultrasound waves promoting the penetration of solvent into the sample matrix and increasing the mass transfer rates of anthocyanins to the extraction solvent (Avhad & Rathod, 2015). In addition, UAE processing might aid the movement of the anthocyanins into the solvent because the ultrasonic power broke down the plant's cell walls (Ramic et al., 2015).

Table 4
Identification of anthocyanins extracted from BWP by two different extraction methods using UPLC–DAD–MS/MS.

Anthocyanins	Retention time (min)	Molecular ion (m/z)	Fragment ion (m/z)	UAE	CSE
Delphinidin-3-*O*-glucoside	3.5	465	303	+	+
Delphindin-3-*O*-arabinoside	3.8	435	303	+	+
Petunidin-3-*O*-glucoside	4.1	479	317	+	+
Cyanidin-3-*O*-arabinoside	4.2	419	287	+	+
Cyanidin-3-*O*-glucoside	4.3	449	287	+	+
Malvidin-3-*O*-glucoside	4.7	493	331	+	+
Malvidin-3-*O*-arabinoside	5.2	463	331	+	+

+, detectable in extract.
UAE: Ultrasound-Assisted Extraction. CSE: Conventional Solvent Extraction.

The UAE method was more efficient than the conventional method in terms of both shortened time and increased yield. Chen et al. (2007) found that UAE was more efficient and rapid when used to extract TA from red raspberry: the CSE took 53 min to yield 35.1 mg of TA from 100 g of red raspberry samples, while UAE took only 3.3 min to get 34.5 mg of TA. Song et al. (2014) found that yield of flavonoids by UAE from pine needles was higher than by CSE: UAE yielded 28.7 rutin equivalents mg/g, but CSE yielded only 22.7 rutin equivalents mg/g.

3.5. Anthocyanins composition analysis by UPLC–DAD–MS/MS

The composition of the anthocyanins in both the UAE and CSE extracts from BWP were identified by HPLC–DAD–MS/MS. The result demonstrated that the same seven anthocyanins, namely delphinidin-3-*O*-glucoside, delphindin-3-*O*-arabinoside, petunidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside and malvidin-3-*O*-arabinoside, were isolated by the two different extraction methods (Table 4) and that the different extract methods did not change the anthocyanins profile.

It is well known that different berries contain different anthocyanins. Nineteen anthocyanins were identified in crowberry, Korean black raspberry, mulberry and strawberry cultivated in Korea (Bae et al., 2015). Twelve anthocyanins were separated, and ten of them identified, in chagalapoli (Ardisia *compressa* K.) fruit cultivated in Mexico (Elvia et al., 2015). Anthocyanins were the glycosides of cyanidin, pelargonidin, peonidin, delphinidin, malvidin, or petunidin. An analysis of 24 wild and cultivated berry species showed that blueberry had the richest content of peonidin glycosides (Veberic, Slatnar, Bizjak, Stampar, & Mikulic-Petkovsek, 2015). Overall, blueberries are one of the richest dietary sources of anthocyanins. Others have been identified 16 anthocyanins in *Vaccinium myrtillu* (Paes, Dotta, Barbero, & Martinez, 2014) and 15 anthocyanins in *Vaccinium angustifolium* (Nicoue, Savard, & Belkacemi, 2007) blueberry extracts. In our investigation of Blueberry Wine Pomace (BWP), we only found 7 anthocyanins in the BWP, all of which have been previously found in blueberry (Muller et al., 2012). The other type anthocyanins may have been fully access blueberry wine during wine-making.

4. Conclusions

In the present work, a Response Surface Methodology (RSM) and a Box–Behnken Design (BBD) were successfully employed to set the optimized parameters for extraction of the bioactive compounds from Blueberry Wine Pomace (BWP). The optimum liquid–solid ratio, extraction temperature and sonication time were predicted for maximum extraction yield of phenolic compounds. In comparison to a Conventional Solvent Extraction (CSE) method, Ultrasound-Assisted Extraction (UAE) resulted in higher recoveries of both TP and TA. Moreover, both UAE and CSE, yielded the same antohcyanins, namely delphinidin-3-*O*-glucoside, delphindin-3-*O*-arabinoside, petunidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside and malvidin-3-*O*-arabinoside, as determined by UPLC–DAD–MS/MS. The study indicated that the use of ultrasonic in the extraction of desired bioactive components from food industry residues was an efficient, economic and environmental extraction technology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.02.094.

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