**Optimization of Solvent and Ultrasound-assisted Extraction for Different Anthocyanin
Rich Fruit and Their Effects on Anthocyanin Compositions**

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Abstract

Conventional solvent extraction (CE) and ultrasound-assisted extraction (UE) were systematically optimized for fruit (blueberries, cherries, and red pear peels) with different anthocyanin compositions to pursue high recovery of polyphenols and anthocyanins with high antioxidant activity in this study. The effects of the extraction methods and conditions on anthocyanin compositions in different fruit were also analyzed by HPLC. The optimum were identified as: 60% methanol, 50 °C, 1 h using CE or 70% methanol, 30 °C, 20 min using UE for blueberries; 60% ethanol, 70 °C, 1 h using CE or 80% ethanol, 30 °C, 20 min using UE for cherries; 60% methanol, 50 °C, 1 h using CE or 60% ethanol, 30 °C, 60 min using UE for red pear peels. From analysis, ultrasound specifically enhanced the extraction of total monomeric anthocyanins from fruit by dissociating polymeric anthocyanins in less polar solvent system. HPLC analysis revealed that both extraction methods and conditions altered the amount of specific anthocyanin compounds in fruit extracts, including delphinidin, cyanidin, petunidin, pelargonidin, peonidin, or malvidin with different sugar moiety. Therefore, different conditions for CE and UE might be implemented for specific fruit with different anthocyanin compositions for maximizing the recovery of anthocyanins.

Keywords: *Polyphenols, anthocyanins, conventional solvent extraction, ultrasound-assisted extraction, anthocyanin composition, HPLC analysis*

1. Introduction

Anthocyanins are a group of polyphenolic flavonoids abundant in fruit, vegetables, and flowers, and contribute to their red, purple and blue colors. The potential health benefits of anthocyanins for preventing certain chronic diseases have been well studied (Zafra-Stone et al., 2007). Considering their nontoxicity and high biocompatibility to human body (Glei et al., 2003), anthocyanins drew increasing attention from food manufacturers and researchers for their usage as natural pigments and antioxidants.

The chemical structure of anthocyanins is a glycoside derivative containing an anthocyanidin (aglycon base in the form of flavylum), different sugar moiety, and possible acylation groups. There are six common anthocyanidins classified by different substitution groups on ring B, including delphinidin (Dp), cyanidin (Cy), petunidin (Pt), pelargonidin (Pg), peonidin (Pn), and malvidin (Mv) (Martin Bueno et al., 2012). As a fact, the composition of anthocyanins vary among fruit. For examples, blueberries contain all five anthocyanidins based anthocyanin except Pg; cherries possess Cy, Pg, and Pn (Wu et al., 2006); for the anthocyanins in red pear peels, mainly Cy and some of Pn based anthocyanins were found (Steyn, Holcroft, Wand, & Jacobs, 2004). As the main route to get the anthocyanin pigments from fruit, the applied solvent extraction conditions (solvent type and concentration, pH, temperature, time, etc.) have been found directly impacting the chemical structure, concentration, and antioxidant activity of anthocyanins in the resulting extracts (Wrolstad et al., 2005; Ignat, Volf, & Popa, 2011). Therefore, it is essential to identify the optimal extraction conditions for each fruit with different anthocyanin compositions, and to maximize the recovery of polyphenols and anthocyanins in the fruit extracts considering variations among fruit species.

This study attempted to identify the optimal extraction conditions for two popular extraction methods, conventional solvent extraction (CE) and ultrasound-assisted extraction (UE). CE method was the traditional solvent extraction method for extracting polyphenols and anthocyanins. The solvents commonly used include methanol, ethanol, and acetone with the concentration of 60-80% for extracting complex structures of anthocyanins with different polarities (Deng, Penner, & Zhao, 2011). Turkmen, Sari, & Velioglu (2006) reported great difference on the extraction efficiency of polyphenols from black and mate teas due to the solvent type they used. A 1% of weak acid, such as acetic acid, is usually added together to avoid the breakage of aromatic acyl acid linkages and/or aliphatic dicarboxyl acyl groups in anthocyanins (Delgado-Vargas, Jiménez, & Paredes-López, 2000). Extraction time and temperature are alternative critical factors for potentially improving CE method (Spigno & De Faveri, 2007; Pinelo et al., 2007). Higher temperature and longer time usually were used to increase the amount of extractable anthocyanins. However, the drawback of this method is the potential structure modifications and/or degradations of bioactive compounds in the extracts under high heat and long-time extraction, inducing low extraction efficiency of stable anthocyanins (Ignat et al., 2011).

Ultrasound, an oscillating sound pressure wave with a frequency over 20 kHz, was highlighted to facilitate the extraction by increasing the mass transfer between solvent and plant material (Garcia-Salas, Morales-Soto, Segura-Carretero, & Fernández-Gutiérrez, 2010; Mason, Chemat, & Vinatoru, 2011). UE method promotes solvent penetration and mass transfer, thus reducing the chemical usage and extraction temperature, increasing extraction rate and yield, and saving cost as well (Viot, Tomao, Le Bourvellec, Renard, & Chemat, 2010). Although a few studies have attempted to develop optimal UE conditions, only one typical plant material was

evaluated in most studies, such as coconut shells (Rodrigues & Pinto, 2007), annatto seeds (Yolmeh, Najafi, & Farhoosh, 2014), wine lees (Tao, Wu, Zhang, & Sun, 2014), and pomegranate (Tabaraki, Heidarizadi, & Benvidi, 2012). High performance liquid chromatography (HPLC) for separation is a powerful tool for identifying anthocyanin compositions in the fruit (Chen et al., 2007; Yue, Shao, Yuan, Wang, & Qiang, 2012; Bai, Zhang, & Ren, 2013). For examples, a study applied HPLC analysis to monitor the influence of processing conditions (conventional heating and ohmic heating) on blueberry anthocyanin color stability (Sarkis, Jaeschke, Tessaro, & Marczak, 2013). Another study evaluated the stability of certain individual anthocyanins in pomegranate juice when subjected to pasteurization and clarification processes (Turfan, Türkyılmaz, Yemiş, & Özkan, 2011). However, few paper simultaneously investigated the possible impact of extraction methods and conditions on the amount and composition of anthocyanins for fruit with different anthocyanin compositions.

This study was to systematically investigate the optimal extraction conditions of CE and UE methods for maximizing the recovery of polyphenols and anthocyanins in three anthocyanin rich fruit with different anthocyanin compositions (blueberries, cherries, and red pear peels) through two combined experimental designs (Taguchi design and completely randomized two-factorial design). The effect of the extracting methods and conditions on anthocyanin compositions in the tested fruit was investigated by HPLC. This study would provide new insights into the impact of extraction methods and conditions on anthocyanin compositions in different anthocyanin rich fruit, and give guidelines on the use of specific CE and UE conditions for particular individual anthocyanin.

2. Material and Methods

2.1. Fruit materials

Fresh blueberries (*Vaccinium Cyanococcus*), sweet cherries (*Prunus avium*), and red pears (*Red D'Anjou*) were purchased from a local market (Corvallis, OR, U.S.A.). Fruit were selected for uniform size and similar maturity, and stored in a 4 °C cooler for extraction within two days.

2.2. Chemical reagents

Folin-Ciocalteu's phenol reagent and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), acetic acid and L-ascorbic acid were from Avantor Performance Materials (Center Valley, PA, U.S.A), and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (95%) was from Alfa Aesar (Ward Hill, MA, U.S.A). Hydrochloric acid, sodium acetate, potassium chloride, sodium carbonate, and trifluoroacetic acid (TFA) were from EMD Chemicals (Gibbstown, NJ, U.S.A.). ACS grade methanol, ethanol, and acetone were from VWR (Radnor, PA, U.S.A.). HPLC grade water and methanol were from EMD Chemicals (Gibbstown, NJ, U.S.A.). Anthocyanin external standards were purchased from PhytoLab (Secaucus, New Jersey, U.S.A.).

2.3. Preparation of fruit samples for extraction

Anthocyanins are located at the mature cell membrane of fruit. For blueberries and cherries, both flesh and peels contain high amount of anthocyanins so that whole fruit were used for the extraction. Whereas, for red pears, almost all anthocyanins are accumulated in the peels (Li & Cheng, 2009), thus only peels were used here.

Approximately 50 g of fruit samples were finely ground in a stainless blender (Waring Products, Torrington, CT, U.S.A.) with liquid nitrogen. Three grams of fruit powders were collected for the following extraction (Wu, Frei, Kennedy, & Zhao, 2010).

2.4. Extraction procedures

Fruit powders and prepared solvents (combinations of different types and concentrations of solvents are shown in Tables 1 and 2) were mixed at a 1:10 solid to solvent ratio in 50 mL centrifuge tubes (VWR, PA, U.S.A.). The mixtures were immersed in a water bath (Precision, VA, U.S.A.) or a 30/40 kHz and 185 W of ultrasonic water bath (Branson B-220H, SmithKline Co., PA, U.S.A.), respectively, at given temperatures and times (Tables 1 and 2).

The obtained extracts were centrifuged (International Equipment, MA, U.S.A.) at 10,000 g for 10 min at 4 °C and filtrated through Whatman No.1 filter paper. Filtrates were evaporated through a vacuum rotary evaporator (Brinkmann Instruments, NY, U.S.A.). Concentrated extracts were then diluted into 25 mL volumetric flask with distilled (DI) water to obtain an appropriate original dilution factor for measurements. Diluted extracts were stored at a -80 °C freezer (VWR, PA, U.S.A.) until analysis.

2.5. Analysis of total phenolics content (TPC), total monomeric anthocyanin (TMA), and DPPH radical scavenging activity (DPPH)

TPC was determined using the Folin-Ciocalteau assay (Singleton, & Rossi, 1965). Briefly, 0.5 mL of diluted extract or 0.5 mL of 0.1, 0.3, 0.5, and 0.7 mg/mL gallic acid solution (used as standard) was mixed with 0.5 mL of Folin-Ciocalteau reagent and 7.5 mL of DI water, respectively. The mixtures were vortexed, kept in dark at room temperature for 20 min, and then transferred into a 40 °C water bath with 3 mL addition of 20% sodium carbonate (w/v) for another 20 min. Samples were immediately cooled in an ice bath for 3 min, and the absorbance of samples was measured at 765 nm using a Shimazu UV160U spectrometer (Shimazu Corp., Kyoto, Japan). Results were expressed as gallic acid equivalent (GAE) mg/g fresh material (FW).

TMA was determined using the pH differential method (Wrolstad et al., 2005). The aqueous extracts were appropriately diluted with 0.025 mol/L potassium chloride buffer (pH=1.0) and 0.4 mol/L sodium acetate buffer (pH=4.5), respectively. The mixture was homogenized at room temperature for 15 min. The absorbance difference between pH 1.0 and pH 4.5 for the diluted extracts was determined at both $\lambda_{\text{vis-max}}$ and 700 nm, respectively. TMA value was then calculated (Wrolstad et al., 2005). The results were expressed as cyanidin 3-glucoside equivalent (Cy-3-glu) mg/100 g FW.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to measure free radical scavenging capacity of the extracts (Brand-Williams, Cuvelier, & Berset, 1995). One mL of diluted extracts and 0.005, 0.01, 0.05, and 0.1 mg/mL of ascorbic acid solution (used as standard) were mixed with 3 mL of methanol solubilized DPPH reagent. Mixtures were vortexed, kept in the dark for 20 min, and the absorbance of samples would then read at 517 nm. Results were reported as ascorbic acid equivalents (AAE) mg/g FW.

2.6. Characterization of anthocyanins

2.6.1. Percent polymeric color (PPC)

PPC could interpret the proportion of polymeric anthocyanin compounds with more stable structure by forming complex in comparison with the monomeric anthocyanins. PPC was calculated as the polymeric color divided by color density (Wrolstad et al., 2005), i.e., the ratio of polymerized anthocyanins in total anthocyanins.

$$\text{PPC} = (\text{polymeric color} / \text{color density}) \times 100\% \quad (3)$$

The polymeric color and color density were determined using the method of Cao et al. (2011). Briefly, 0.2 mL of 0.2 g/mL potassium meta-bisulfite was added into 2.8 mL of diluted

sample (bleached sample), and 0.2 mL of DI water was added to 2.8 mL of diluted sample as the control sample. After equilibrating for 30 min at ambient conditions, the absorbance of the samples was evaluated at wavelength of 420 nm, 700 nm, and $\lambda_{\text{vis-max}}$ (500-510 nm), respectively.

Color density and polymeric color were all calculated using the formula:

$$[(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{\lambda_{\text{vis-max}}} - A_{700\text{nm}})] \times \text{dilution factor} \quad (4)$$

where the color density was tested on the control sample, and the polymeric color was tested on the bleached sample using same formula.

2.6.2. HPLC characterization and quantitation

Anthocyanin compositions in blueberry, cherry and red pear peel extracts were determined using a Shimadzu liquid chromatograph system (Shimadzu, Kyoto, Japan) monitored by a LC-10A VP control system equipped with two LC-10AS liquid chromatograph, one SPD-20A UV/VIS detector, and a remote controlling computer with Shimadzu EZStart 7.2.1 SP1 software. The procedures from Bordonaba, Crespo, & Terry (2011) were applied with some modifications. Briefly, a 0.22 μm syringe was used to manually inject 10 μL of final diluted samples into a C18 column (250 x 4.60 mm, 5 μm particle size) (Restek Co., PA, U.S.A.). The dilution of extracts followed blueberry (1:25 v:v), cherry (1:5 v:v), and red pear peels (no dilution) for fitting proper absorbance in profiles. Mobile phases utilized (A) 2% (v/v) acetic acid in HPLC grade water, and (B) 2% TFA (v/v) in HPLC grade methanol. The gradient conditions were: 0-10 min, 2-20% B; 10-20 min, 20 – 25% B; 20-25 min, 25-35% B; 25-35 min, 35-75% B; 35-45 min, 75% B. Flow rate was 1 mL/min.

Eluted compounds from the fruit extracts were all detected using 540 nm. The presence and quantity of each anthocyanin were determined by comparing the peak area with external

standards, Cy-3-gal, Cy-3-glu, and Cy-3-rut. Peak identifications of the compounds with no external standard were conducted by consulting previous literatures (Bordonaba et al., 2011; Ngo & Zhao, 2009; Wu et al., 2006) and quantified using external standards that were closest to their chemical structure; Dp-3-gal, Dp-3-glu, and Dp-3-ara with Delphinidin-3-glucoside, Pt-3-gal, Pt-3-glu, and Pt-3-ara with Petunidin-3-glucoside, Pn-3-glu and Pn-3-ara with Peonidin and Mv-3-gal with cyanidin-3-galactoside and Mv-3-glu and Mv-3-ara with Cyanidin-3-glucoside. **This method was confirmed by spike recoveries**, in which a known amount of anthocyanin was added to a sample and the resulting concentration was determined. All spike recoveries fell within 100% $\pm$ 5%.

2.7. Experimental design

For optimizing the extraction conditions for each method, two experimental designs were consecutively applied in this study. Taguchi design **was** firstly applied to select the two most important contributing factors among the commonly applied three factors, assuming that there was no interaction between factors. Taguchi design offered a simple and systematic approach to reduce the number of treatment combinations when multiple factors were considered, **and it could** inform the contribution of individual treatment factor (Karabas, 2014). This information would be especially valuable for industrial or scale-up extraction process, considering less number of factors critically affecting quality characteristics of obtained extracts. For CE, the type of the organic solvent (methanol, ethanol and acetone), concentration of the solvent in 1% acetic acid solution (v/v/v) (60, 70 and 80%), and temperature (50, 60 and 70 °C) were chosen based on the previous studies (Bridgers, Chinn, & Truong, 2010; Kapasakalidis, Rastall, & Gordon, 2006). **The extracting time was 1 h for all combined extracting conditions.** For UE, different ultrasound

treated times (20, 40, and 60 min) were considered along with the solvent types (methanol, ethanol and acetone) and concentrations of the solvents in 1% acetic acid solution (v/v/v) (60, 70 and 80%) at water bath temperature of 30 ± 0.5 °C (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008; Mané et al., 2007; Spigno, Tramelli, & De Faveri, 2007). The temperature effect was not considered in UE method based on our preliminary studies, showing that UE with continuous high heat processing could reduce the anthocyanin content in the extract, probably caused by the severe extracting condition along with the severe degradation of anthocyanin compounds (data not shown).

In Taguchi design, two parameters were used to determine the mostly contributing factors. The first parameter K_{ij} was the average value of each measured functional parameter in level j ($j=1, 2, 3$) of each factor i ($i=A, B, C$) and expressed as (Jung & Zhao, 2011):

$$K_{ij} = \frac{1}{N_i} \sum_{u=1}^{N_i} y_{i,j,u} \quad (5)$$

where i represented the factor A, B and C; j represented the level 1, 2 and 3; N_i was the number of trials for each factor; and $y_{i,j}$ was the measured responses of factor i at level j .

The second parameter, R_i , was the difference between the highest and lowest values of K_{ij} to determine the most contributed factor, and expressed as:

$$R_i = (K_{ij})_{\max} - (K_{ij})_{\min} \quad (6)$$

where $(K_{ij})_{\max}$ and $(K_{ij})_{\min}$ indicated the highest and lowest values of the measured values under each factor, respectively. The factor with the larger R_i value was considered as more contributing factor in the processing. The two mostly contributing factors for extraction were chosen considering both the rank of R_i values for each factor and the factors showing significance ($P < 0.05$) by ANOVA analysis.

Based upon the results from Taguchi design, a 2x2 completely randomized factorial design was then applied to optimize the extraction conditions, in which the possible interactions between the two selected factors were considered. And high contents of TPC, TMA, DPPH, and PPC values in the extracts were targeted as well. HPLC analysis was further conducted to investigate the effect of different extraction methods and conditions on the anthocyanin compositions in each fruit extract.

2.8. Statistical analysis

All experiments were conducted in triplicates. For a 2x2 completely randomized factorial design, the analysis of variance (ANOVA) was conducted to evaluate significant effect of the individual factor and their interactions. The multiple comparison *post-hoc* least significant difference test (LSD) was used to examine the difference among the levels of extraction factors at $P < 0.05$. ANOVA and LSD were performed using SAS 9.2 (SAS Institute Inc., NC, U.S.A.). Principal component analysis (PCA) was conducted to show classification of the individual anthocyanins in blueberry extracts which proved containing up to 14 identified compounds in HPLC analysis and the relationship of the extraction variables in the extraction conditions was determined using XLSTAT from Addinsoft (New York, NY, U.S.A.).

3. Results and discussions

3.1. Mostly contributing extraction factors based on the analysis of Taguchi design

Taguchi design successfully selected two main contributing factors among the three treatment factors for each extraction method (Tables 1 and 2). For CE (Table 1), type of solvent (factor A) and temperature (factor C) were identified as the two main contributing factors on

TPC and TMA for all fruit and DPPH for red pear peels. Type of solvent (factor A) and solvent concentration (factor B) were most important for blueberries, and temperature (factor B) and solvent concentration (factor C) were most important for cherries. Further analysis through ANOVA (data not shown) provided a more convincing evidence for the significant effect of the type of solvent and temperature on different measurements (marked as asterisk in Table 1). Hence, two treatment factors, the type of solvent and temperature, were selected for optimizing the conditions of CE method using a 2x2 completely randomized factorial design.

In respect to UE (Table 2), for blueberries, type of solvent (factor A) and concentration (factor B) appeared to be the two main contributing factors on both TPC and DPPH, whereas solvent concentration (factor B) and time (factor C) were on TMA. For cherries, type of solvent (factor A) and solvent concentration (factor B) were identified as the two main contributing factors on both TMA and DPPH, but solvent concentration (factor B) and time (factor C) were on TPC. For red pear peels, type of solvent (factor A) and time (factor C) were represented as the two main contributing factors on TPC, TMA and DPPH. Through ANOVA analysis (data not shown), it was found that the solvent concentration was only significant ($P<0.05$) on TMA in blueberries, whereas solvent concentration and type of solvent significantly ($P<0.05$) affected TPC and TMA of cherries, respectively. In red pear peels, no significant factor was detected (marked as asterisk in Table 2). For following two-factorial design and analysis, therefore, the type and concentration of solvent were selected for blueberries and cherries, whereas type of solvent and extraction time were chosen for red pear peels.

3.2. Optimal level of significant treatment factors identified from the 2x2 completely randomized factorial design

Two factors at three levels chosen from the Taguchi analysis were investigated using a 2x2 completely randomized factorial design to determine the optimal CE and UE conditions for three fruit materials. In addition to TPC, TMA, and DPPH of the fruit extracts, PPC, indicating proportion of polymeric anthocyanins in the extract, was also measured to investigate the effect of extraction conditions on anthocyanin polymerization (Vatai, Škerget, & Knez, 2009). PPC has been previously analyzed for processed blueberries (Brownmiller, Howard, & Prior, 2008) and strawberry purees (Holzwarth, Korhummel, Kammerer, & Carle, 2012).

Based on ANOVA analysis (data not shown), no significant interactive effect between the treatment factors on TPC, TMA, DPPH, and PPC was observed in the three types of fruit extracts using either CE or UE method. Hence, only the mean values of each treatment level within the independent factor showing significant effect on the different measurements were reported based on *post-hoc* LSD results (Fig. 1). For CE-blueberries, methanol extraction significantly ($P<0.05$) enhanced TPC and TMA in comparison with ethanol and acetone, respectively. Similarly, Deng et al. (2014) found that methanol extracts contain higher TPC in blueberry leaf extracts; Cheok, Chin, Yusof, & Law (2011) also proved that methanol is the most effective solvent for phenolics recovery from hull of mangosteen (*Garcinia mangostana* Linn) in comparison with ethanol, acetone, isopropanol, ethyl acetate, and distilled water. Previous study also indicated that acetone significantly ($P<0.05$) enhanced PPC in the extract, probably because less polar polymeric anthocyanins was more readily to be extracted in the least polar solvent mixture containing acetone (polarity of the solvents is in the order of acetone < ethanol < methanol < water) (Meskin, Bidlack, & Randolph, 2008). Consistently, de Souza et al. (2014) claimed that combined methanol and acetone can enhance polyphenol recovery in the extract from Brazilian berries. For UE-blueberries, methanol extract also obtained significantly ($P<0.05$) higher TPC

and DPPH. In addition, solvent concentration at 70% and 80% significantly ($P<0.05$) enhanced TMA. This result contradicted with the result using CE, in which more polar TMA was recovered from lower concentrations of solvent in the mixture with high polarity (Ju & Howard, 2003). This difference might be caused by dissociation promotion of the polymeric anthocyanins in less polar solvent mixture, thus obtaining more TMA in the extract.

For CE-cherries, the extracts using ethanol or at 70 °C showed significantly ($P<0.05$) higher TPC, TMA, and DPPH than at other conditions. PPC presented significantly ($P<0.05$) higher when extracting at 50 °C, since higher temperature could dissociate the polymeric anthocyanins to smaller structures. Pinelo, Fabbro, Manzocco, Nuñez, & Nicoli (2005) also supported 50 °C providing the highest polyphenol recovery from grape byproducts. For UE-cherries, TPC was significantly ($P<0.05$) higher in methanol or ethanol extracts, whereas higher TMA was found in the least polar acetone extract. This result was also inconsistent with that polar TMA can be more recovered in more polar solvent system (Ju & Howard, 2003). Likewise, **it was all induced by the ultrasound energy that potentially promoted polymeric anthocyanins in acetone.**

For CE-red pear peels, methanol extracts showed significantly ($P<0.05$) higher TMA and DPPH than acetone extracts, whereas ethanol and acetone extracts had significantly ($P<0.05$) higher TPC. Interestingly, PPC was **increasing** with extended extraction time in UE-red pear peels. This result was contradicted with other fruit using UE, in which TMA was enhanced by ultrasound energy. This difference could be related to the different cell wall structures among fruit. The ultrasound could enhance penetration for solvent to the firmer cell wall structure in pears through thick wax cuticle layer, thus assisting the extraction of polymeric anthocyanins without dissociating the anthocyanins. Hence, it might be concluded that UE is recommended for materials with firm cell wall structure for effectively disrupting the cells.

Based on the above results and the criteria used for selecting the optimal extraction conditions (high extraction efficiency and lower level of solvent concentration, temperature and time for saving cost, time, and energy), the optimal extraction conditions were set as: blueberries, 60% methanol at 50 °C for 1 h using CE and 70% methanol at 30 °C for 20 min using UE; cherries, 60% ethanol at 70 °C for 1 h using CE and 80% ethanol at 30 °C for 20 min using UE; red pear peels, 60% methanol at 50 °C for 1 h using CE and 60% ethanol at 30 °C for 1 h using UE.

3.3. Effect of different extraction conditions on anthocyanin compositions

Anthocyanin compositional chromatogram for different fruit extracts are provided in Supplementary Fig. 1. Anthocyanin compositions varied significantly depending on the type of fruit, in which blueberries contained the most various anthocyanidins than cherries and red pear peels. Specifically, blueberry extracts contained: (1) delphinidin-3-galactoside (Dp-3-gal), (2) delphinidin-3-glucoside (Dp-3-glu), (3) cyanidin-3-galactoside (Cy-3-gal), (4) delphinidin-3-arabinoside (Dp-3-ara), (5) cyanidin-3-glucoside (Cy-3-glu), (6) petunidin-3-galactoside (Pt-3-gal), (7) cyanidin-3-arabinoside (Cy-3-ara), (8) petunidin-3-glucoside (Pt-3-glu), (9) petunidin-3-arabinoside (Pt-3-ara), (10) peonidin-3-glucoside (Pn-3-glu), (11) malvidin-3-galactoside (Mv-3-gal), (12) peonidin-3-arabinoside (Pn-3-ara), (13) malvidin-3-glucoside (Mv-3-glu), and (14) malvidin-3-arabinosides (Mv-3-ara). Cherry extracts were composed of three anthocyanins: (1) Cy-3-glu, (2) cyanidin-3-rutinoside (Cy-3-rut), and (3) an unknown compound. Cy-3-rut was only found in cherry extracts. Red pear peel extracts contained two anthocyanins: (1) Cy-3-gal and (2) Cy-3-glu. These results were similar to those found by Barnes, Nguyen, Shen, & Schug (2009). They identified 25 individual anthocyanins in blueberries with similar anthocyanidin

compositions. The presence of Cy-3-rut was also reported as a main compound in dark colored cherries by Cao et al. (2015). For red pears, Cya-3-gal was identified as the major individual anthocyanins (Dussi, Sugar, & Wrolstad, 1995).

ANOVA results showed the effect of independent factors and their interactions on the anthocyanin compositions in different fruit extracts using CE or UE method (Table 3). For CE-blueberries, solvent types had significant ($P<0.05$) effect on quantities of 11 different anthocyanins except for Dp-3-glu, Pt-3-glu, and Pn-3-glu. Temperature had significant ($P<0.05$) effect on quantities of Dp-3-gal, Dp-3-ara, Cy-3-glu, Mv-3-gal, Pn-3-ara, Mv-3-glu, and Mv-3-ara. Significant ($P<0.05$) interactive effects between solvent type and temperature were found on Dp-3-gal, Cy-3-gal, Dp-3-ara, Cy-3-glu, Pn-3-glu, Mv-3-gal, Pn-3-ara, Mv-3-glu, and Mv-3-ara. For UE-blueberries, solvent types had significant ($P<0.05$) effect on 9 different anthocyanins, excluding Dp-3-glu, Cy-3-glu, Pt-3-gal, Cy-3-ara, and Pt-3-ara. Solvent concentrations significantly ($P<0.05$) affected Dp-3-gal, Dp-3-glu, Cy-3-gal, Pn-3-glu, Mv-3-gal, Pn-3-ara, and Mv-3-ara. Significant ($P<0.05$) interactive effects between solvent type and concentration were found on Cy-3-gal, Pt-3-ara, Pn-3-glu, Mv-3-gal, Pn-3-ara, Mv-3-glu, and Mv-3-ara. For CE-cherries, Cy-3-glu was significantly ($P<0.05$) affected by solvent type, whereas solvent type, temperature, and their interaction had significant ($P<0.05$) effect on Cy-3-rut. For UE-cherries, Cy-3-rut was significantly ($P<0.05$) affected by solvent type, concentration, and their interaction. For CE-red pears, solvent type had significant ($P<0.05$) effect on both Cy-3-glu and Cy-3-rut, whereas the significant ($P<0.05$) interaction between solvent type and temperature was found on Cy-3-glu. For UE-red pear peels, only solvent type had significant ($P<0.05$) effect on Cy-3-gal. Hence, ANOVA analysis demonstrated different susceptibility of individual anthocyanin compound to the extraction methods and conditions.

Multiple comparison *post-hoc* LSD test results are reported in Table 4 to illustrate the effect of extracting methods and conditions (i.e., combined two levels of each factor) on the quantity of individual anthocyanins in blueberry, cherry, and red pear peel extracts. For CE-blueberries, Dp-3-gal, Cy-3-gal, Dp-3-ara, Cy-3-glu, Pn-3-glu, Mv-3-gal, Pn-3-ara, Mv-3-glu, and Mv-3-ara were significantly ($P<0.05$) higher when using acetone at 50 °C, acetone at 50 °C and 70 °C, methanol at 50 °C, acetone at 50 °C, acetone and methanol at 70 °C, methanol at 70 °C, acetone at all three temperatures and methanol at 70°C, or acetone at 50°C, respectively, in comparison with other combined levels of extractions. These results demonstrated that acetone promoted the extraction of Dp-3-gal, Cy-3-gal, Cy-3-glu, Pn-3-glu, Pn-3-ara, and Mv-3-ara, whereas methanol resulted in higher extraction efficiency on Dp-3-ara, Pn-3-glu, Mv-3-gal, Pn-3-ara and Mv-3-glu. For CE-cherries, Cy-3-rut was significantly ($P<0.05$) higher with methanol at 60 °C, and for red pear peels, Cy-3-gal was detected significantly ($P<0.05$) higher with ethanol at 50 °C. These differences on the optimal extraction conditions might be related to the different polarity of each anthocyanin compound toward solvent. For UE-blueberries, Cy-3-gal, Pn-3-glu, Mv-3-gal, Pn-3-ara, Mv-3-glu, and Mv-3-ara were significantly ($P<0.05$) higher when extracted using 60 to 80% acetone, 70 and 80% methanol, 70% acetone, 60% methanol, 70% acetone, and 70% methanol, respectively, in comparison with other combined levels of extractions. Acetone enhanced the extraction of Cy-3-gal, Mv-3-gal, and Mv-3-glu, whereas methanol was more suitable for extracting Pn-3-glu, Pn-3-ara, and Mv-3-ara. The 70% acetone and ethanol extracted higher Mv-3-gal and Mv-3-glu. For UE-cherries, 60% ethanol or 60 and 70% methanol significantly ($P<0.05$) increased Cy-3-rut, compared with other treatments. These results demonstrated that the extracting conditions selectively altered anthocyanin compositions in the extract, thus the

exact extracting conditions should be strictly selected based on the individual anthocyanin compounds of interest.

To further interpret the correlation of anthocyanin compositions with the extraction conditions used for the fruit with complex anthocyanin composition, PCA used for blueberry extracts obviously proved that specific extraction conditions favor different groups of anthocyanin compounds (Fig. 2). The first two factors in PCA accounted for 66.43% of the total variance ($F1=41.64\%$, $F2=24.79\%$). Specifically, along $F1$ showed two groups, one along the negative axis and the other along the positive axis. This was characterized by a greater number of anthocyanins and occurred because UE produced a lower number of overall anthocyanins in the resulting extracts. Of these two main groups, CE-1, CE-2, CE-3 and CE-5 were characterized by Cy-3-glu, Mv-3-ara, Pn-3-ara, and Pt-3-ara, and CE-9, UE-1, UE-2 and UE-3 were characterized by Cy-3-ara, Dp-3-ara, Mv-3-gal, Mv-3-glu and Pt-3-glu, respectively. CE-7, UE-4, UE-5, UE-6 and UE-9 were not characterized by any specific groupings of anthocyanins, but were found to contain similar amounts of anthocyanins as they were found on the PCA graph in between the anthocyanin vectors. CE-4, CE-6 and CE-8 contained lower concentrations of the anthocyanins due to their closer proximity to the (0, 0) point in the PCA figure. UE-7 was characterized by Dp-3-glu and Pt-3-gal. UE-8 was characterized by Pn-3-glu. Therefore, it can be seen that depending on the anthocyanin(s) of interest, there were different extraction methods and conditions that may be preferred over others.

4. Conclusion

Both conventional solvent extraction and ultrasound-assisted extraction methods were optimized to enhance the recovery of polyphenols, anthocyanins, and antioxidant activity in the

extracts from blueberries, cherries, and red pear peels containing different anthocyanin compositions. The extracting conditions for each method were systematically optimized through the Taguchi analysis and 2x2 completely randomized two-factorial design. It was found that UE method dissociated polymeric anthocyanins without further degradation of the monomeric anthocyanins, thus enhancing total monomeric anthocyanin content in blueberry and cherry acetone extracts. The ultrasound assistance might be more suitable for materials with firm cell wall structure for effective cell disruptions, thus allowing the use of low concentration of solvent and lower temperature for anthocyanin extraction. HPLC analysis revealed that extraction methods and conditions altered the specific anthocyanin compositions in fruit extracts. Depending on the individual anthocyanins of interest, therefore, different extracting methods and conditions should be applied. Other extraction assisting technologies, such as microwave and high hydrostatic pressure may be evaluated to examine the optimal extraction method and conditions on different anthocyanin rich fruit in future work. Additionally other types of anthocyanin rich fruit with different anthocyanin compounds may also be investigated using the developed optimal extraction conditions to compare with the results obtained from this study.

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Table 1 Contribution of each treatment factor on total phenolic content (TPC), total monomeric anthocyanin (TMA), and DPPH radical scavenging activity of blueberries, cherries, and red pear peels using conventional solvent extraction (CE) method

Number of trials	Type of fruits											
	Factors			Blueberries			Cherries			Red pear peels		
	A	B	C	TPC	TMA	DPPH	TPC	TMA	DPPH	TPC	TMA	DPPH
1	Methanol	60	50	2.74	37.96	4.84	1.28	4.12	0.80	1.55	2.23	5.73
2	Methanol	70	60	2.77	46.29	4.61	1.39	6.17	0.90	1.56	1.59	5.95
3	Methanol	80	70	3.05	49.83	4.22	1.31	4.81	0.86	2.14	3.42	6.90
4	Ethanol	60	60	3.28	40.55	2.24	1.46	5.90	0.89	1.16	0.92	5.56
5	Ethanol	70	70	3.51	55.59	4.97	1.46	4.58	0.87	2.00	2.40	6.57
6	Ethanol	80	50	3.01	60.73	1.02	1.42	4.00	0.82	1.92	2.13	6.15
7	Acetone	60	70	3.64	63.09	1.53	1.39	3.10	0.88	2.52	3.01	7.52
8	Acetone	70	50	3.16	53.01	4.68	1.37	2.69	0.84	1.94	1.74	6.33
9	Acetone	80	60	3.08	52.99	5.17	1.40	3.59	0.81	1.85	1.47	6.65
Factor	Levels	Values										
A	Methanol	K _{A1}		2.86 ^b	44.69 ^b	4.55 ^a	1.33 ^a	5.03 ^a	0.86 ^a	1.75 ^b	2.41 ^a	6.19 ^b
	Ethanol	K _{A2}		3.27 ^a	52.29 ^{ab}	2.74 ^b	1.45 ^a	4.83 ^a	0.86 ^a	1.69 ^b	1.82 ^b	6.09 ^b
	Acetone	K _{A3}		3.29 ^a	56.36 ^a	3.79 ^{ab}	1.39 ^a	3.12 ^b	0.84 ^a	2.10 ^a	2.07 ^{ab}	6.83 ^a
B		R _A ⁺		0.44	11.67	1.81	0.12	1.91	0.01	0.41	0.60	0.74
	60	K _{B1}		3.22 ^a	47.20 ^a	2.87 ^b	1.38 ^a	4.37 ^a	0.86 ^a	1.75 ^b	2.05 ^a	6.27 ^a
	70	K _{B2}		3.15 ^a	51.63 ^a	4.75 ^a	1.41 ^a	4.48 ^a	0.87 ^a	1.83 ^{ab}	1.91 ^a	6.28 ^a
	80	K _{B3}		3.05 ^a	54.52 ^a	3.47 ^{ab}	1.37 ^a	4.13 ^a	0.83 ^a	1.97 ^a	2.34 ^a	6.57 ^a
		R _B ⁺		0.17	7.31	1.88	0.03	0.35	0.04	0.22	0.43	0.30
C	50	K _{C1}		2.97 ^b	50.57 ^a	3.51 ^a	1.36 ^a	3.60 ^b	0.82 ^b	1.80 ^b	2.03 ^b	6.07 ^b
	60	K _{C2}		3.04 ^b	46.61 ^a	4.01 ^a	1.42 ^a	5.22 ^a	0.87 ^a	1.52 ^c	1.33 ^c	6.05 ^b
	70	K _{C3}		3.40 ^a	56.17 ^a	3.57 ^a	1.38 ^a	4.16 ^{ab}	0.87 ^a	2.22 ^a	2.94 ^a	6.99 ^a
		R _C ⁺		0.43	9.56	0.49	0.06	1.62	0.05	0.70	1.62	0.94
Rank ⁺⁺				A(*)>C(*)>B	A>C>B	B>A>C	A>C>B	A(*)>C>B	C(*)>B>A	C(*)>A(*)>B	C(*)>A>B	C(*)>A(*)>B
The two mostly contributing factors				Factor A and C			Factor A and C			Factor A and C		

Factor A, B, and C represent types of solvent, concentration (%) of solvent in 1% acetic acid, and temperature (°C), respectively. Units for TPC, TMA, and DPPH value were gallic acid equivalent mg/g fresh material, cyanidin-3-glucoside equivalent mg/100 g fresh material, ascorbic acid equivalent mg/g fresh material, respectively.

⁺Kij were the average values of each measured parameter from nine treatment set in level j (j=1, 2, 3) of each factor i (i=A, B or C). The same lowercase letter above Kij of each measured parameter indicated no significant difference detected by LSD test among levels for the same factor (P>0.05).

⁺⁺ R_A, R_B, and R_C were the differences between the highest and lowest values of Kij within the same factor i

⁺⁺⁺ Rank was based on the largest to smallest order of R_A , R_B , and R_C value, the factor with asterisk meant significance detected by ANOVA analysis ($P>0.05$)

⁺⁺⁺⁺ The two most contributing factors were selected based on the significance and rank order of R_i .

Table 2 Contribution of each treatment factor on total phenolic content (TPC), total monomeric anthocyanin (TMA), and DPPH radical scavenging activity of blueberries, cherries, and red pear peels using ultrasound-assisted extraction (UE) method

Number of trials	Type of fruits											
	Factors			Blueberries			Cherries			Red pear peels		
	A	B	C	TPC	TMA	DPPH	TPC	TMA	DPPH	TPC	TMA	DPPH
1	Methanol	60	20	2.04	32.42	4.75	1.63	7.54	0.91	1.43	0.62	5.46
2	Methanol	70	40	2.45	57.01	4.76	1.44	6.86	0.86	1.45	0.93	5.85
3	Methanol	80	60	2.62	54.46	4.19	1.44	7.04	0.91	1.50	2.48	5.67
4	Ethanol	60	40	3.00	44.08	2.24	1.56	8.68	0.89	1.48	0.65	5.71
5	Ethanol	70	60	2.04	45.83	4.85	1.37	5.18	0.84	1.73	0.78	5.76
6	Ethanol	80	20	2.48	46.61	1.02	1.44	6.62	0.88	1.53	0.48	5.85
7	Acetone	60	40	3.46	45.33	1.50	1.55	4.55	0.88	1.75	0.82	5.77
8	Acetone	70	20	2.99	48.70	4.60	1.47	5.40	0.88	1.56	0.79	5.51
9	Acetone	80	60	2.47	56.36	5.49	1.34	4.27	0.84	1.70	1.15	5.53
Factor	Levels	Values										
A	Methanol	K _{A1}		2.37 ^a	47.96 ^a	4.57 ^a	1.50 ^a	7.14 ^a	0.89 ^a	1.46 ^b	1.34 ^a	5.66 ^a
	Ethanol	K _{A2}		2.51 ^a	45.51 ^a	2.70 ^b	1.46 ^a	6.83 ^a	0.87 ^{ab}	1.58 ^{ab}	0.63 ^a	5.78 ^a
	Acetone	K _{A3}		2.97 ^a	50.13 ^a	3.86 ^{ab}	1.45 ^a	4.74 ^b	0.86 ^b	1.67 ^a	0.92 ^a	5.60 ^a
B		R _A ⁺		0.60	4.63	1.86	0.05	2.40	0.03	0.21	0.71	0.17
	60	K _{B1}		2.84 ^a	40.61 ^b	2.83 ^b	1.58 ^a	6.92 ^a	0.89 ^a	1.55 ^a	0.70 ^a	5.65 ^a
	70	K _{B2}		2.49 ^a	50.51 ^a	4.74 ^a	1.43 ^b	5.81 ^a	0.86 ^b	1.58 ^a	0.83 ^a	5.71 ^a
C	80	K _{B3}		2.53 ^a	52.48 ^a	3.57 ^{ab}	1.41 ^b	5.97 ^a	0.88 ^{ab}	1.58 ^a	1.37 ^a	5.68 ^a
		R _B ⁺		0.35	11.87	1.91	0.17	1.11	0.03	0.03	0.67	0.06
	20	K _{C1}		2.50 ^a	42.58 ^b	3.46 ^a	1.51 ^a	6.52 ^a	0.89 ^a	1.51 ^b	0.63 ^a	5.61 ^a
C	40	K _{C2}		2.64 ^a	52.48 ^a	4.16 ^a	1.45 ^a	6.60 ^a	0.86 ^a	1.54 ^{ab}	0.91 ^a	5.70 ^a
	60	K _{C3}		2.71 ^a	48.54 ^{ab}	3.51 ^a	1.46 ^a	5.59 ^a	0.88 ^a	1.66 ^a	1.36 ^a	5.74 ^a
		R _C ⁺		0.20	9.91	0.71	0.06	1.01	0.03	0.15	0.73	0.13
Rank ⁺⁺				A>B>C	B(*)>C>A	B>A>C	B(*)>C>A	A(*)>B>C	A>B>C	A>C>B	C>A>B	A>C>B
The two mostly contributing factors				Factor A and B			Factor A and B			Factor A and C		

Factor A, B, and C represented the type of solvent, concentration (%) of solvent in 1% acetic acid, and time (min), respectively.

Units for each TPC, TMA, and DPPH value were gallic acid equivalent mg/g fresh material, cyanidin-3-glucoside equivalent mg/100g fresh material, ascorbic acid equivalent mg/g fresh material, respectively.

⁺Kij were the average values of each measured parameter from nine treatment sets in level j (j=1, 2, 3) of each factor i (i=A, B or C). The same lowercase letter above Kij of each measured parameters indicated no significant difference detected by LSD test among levels for the same factor ($P>0.05$).

⁺⁺ R_A, R_B, and R_C were the difference between the highest and lowest values of Kij within the same factor i (i= A, B or C).

⁺⁺⁺ Rank was based on the largest to smallest order of R_A , R_B , and R_C value, the factor with asterisk meant significance detected by ANOVA analysis ($\alpha=0.05$)

⁺⁺⁺⁺ The two most contributing factors were selected based on the significance and rank order of R_i .

Table 3 Analysis of variance (ANOVA) results ($p=0.05$) for analyzing the effect of independence or interactions among treatment factors on anthocyanin compositions of blueberry, cherry, and red pear peel extracts by using conventional solvent extraction (CE) and ultrasound-assisted extraction (UE) methods

(A) Blueberries														
CE method														
Type of anthocyanin ⁺	Dp-3-gal	Dp-3-glu	Cy-3-gal	Dp-3-ara	Cy-3-glu	Pt-3-gal	Cy-3-ara	Pt-3-glu	Pt-3-ara	Pn-3-glu	Mv-3-gal	Pn-3-ara	Mv-3-glu	Mv-3-ara
Linear terms														
Solvent (SV)	0.0009	0.2189	0.0009	0.0001	0.0001	0.0003	0.0053	0.3054	0.0001	0.5888	0.0001	0.0001	0.0001	0.0001
Temperature (TP)	0.0190	0.5242	0.1601	0.0009	0.0002	0.7101	0.7759	0.5611	0.7703	0.2679	0.0001	0.0176	0.0001	0.0026
Interaction terms														
SV x TP	0.0004	0.8496	0.0013	0.0006	0.0001	0.3049	0.7807	0.4239	0.7553	0.0419	0.0001	0.0001	0.0001	0.0001
UE method														
Linear terms														
Solvent (SV)	0.0001	0.4233	0.0001	0.0001	0.4823	0.5795	0.3896	0.0474	0.2076	0.0001	0.0001	0.0001	0.0001	0.006
Concentration (CT)	0.0311	0.0261	0.0058	0.1089	0.2817	0.1697	0.3440	0.2059	0.0967	0.0001	0.0001	0.0007	0.1087	0.0011
Interaction terms														
SV x CT	0.1438	0.0696	0.0005	0.3592	0.0648	0.6558	0.2550	0.8662	0.0220	0.0001	0.0001	0.0001	0.0001	0.0019
(B) Cherries						(C) Red pear peels								
CE method			UE method			CE method			UE method					
Type of anthocyanin	Cy-3-glu	Cy-3-rut	Type of anthocyanin	Cy-3-glu	Cy-3-rut	Type of anthocyanin	Cy-3-gal	Cy-3-glu	Type of anthocyanin	Cy-3-gal	Cy-3-glu	Type of anthocyanin	Cy-3-gal	Cy-3-glu
Linear terms			Linear terms			Linear terms			Linear terms			Linear terms		
Solvent (SV)	0.0282	0.0001	Solvent (SV)	0.1803	0.0001	Solvent (SV)	0.0068	0.0466	Solvent (SV)			Solvent (SV)	0.0010	
Temperature (TP)	0.0712	0.0001	Concentration (CT)	0.5852	0.0048	Temperature (TP)	0.1878	0.1946	Time (TM)			Time (TM)	0.8764	
Interaction terms			Interaction terms			Interaction terms			Interaction terms			Interaction terms		
SV x TP	0.8122	0.0007	SV x CT	0.0792	0.0001	SV x TP	0.0102	0.5594	SV x TM			SV x TM	0.4917	

⁺ Delphinidin-3-galactoside = Dp-3-gal; Delphinidin-3-glucoside = Dp-3-glu; Cyanidin-3-galactoside = Cy-3-gal; Delphinidin-3-arabinoside = Dp-3-ara; Cyanidin-3-glucoside = Cy-3-glu; Petunidin-3-galactoside = Pt-3-gal; Cyanidin-3-arabinoside = Cy-3-ara; Petunidin-3-glucoside = Pt-3-glu; Petunidin-3-arabinoside = Pt-3-ara; Peonidin-3-glucoside = Pn-3-glu; Malvidin-3-galactoside = Mv-3-gal; Peonidin-3-arabinoside = Pn-3-ara; Malvidin-3-glucoside = Mv-3-glu; Malvidin-3-arabinosides = Mv-3-ara.

Table 4 The effect of different extraction conditions on the individual anthocyanin compounds in blueberry, cherry, and red pear peel extracts by conventional solvent extraction (CE) and ultrasound-assisted extraction (UE) methods

[A] Blueberry												
Factors			CE (µg/g FW)									
Solvent	Temp (°C)	Label	Dp-3-gal	Cy-3-gal	Dp-3-ara	Cy-3-glu	Pn-3-glu	Mv-3-gal	Pn-3-ara	Mv-3-glu	Mv-3-ara	
Acetone	50	CE-1	109.96 a	147.08 a	13.46 d	170.19 a	28.00 ab	45.15 c	507.22 a	23.43 d	123.11 a	
	60	CE-2	86.47 bc	131.39 ab	ND*	133.28 b	12.89 b	30.95 g	477.17 a	20.39 g	98.10 f	
	70	CE-3	92.32 bc	145.67 a	ND	147.47 ab	52.31 a	25.97 h	547.34 a	26.14 b	114.53 b	
Ethanol	50	CE-4	64.69 de	95.69 cd	ND	101.03 c	42.68 ab	182.74 b	12.14 d	16.68 h	85.01 g	
	60	CE-5	86.26 bc	131.09 ab	ND	142.16 b	25.04 ab	36.44 f	463.19 ab	23.39 e	99.94 e	
	70	CE-6	79.23 cd	115.60 bc	59.77 b	21.73 d	ND	39.64 d	359.88 bc	23.02 f	101.53 d	
Methanol	50	CE-7	99.45 ab	136.89 ab	80.10 a	28.16 d	18.99 ab	36.74 e	468.06 a	26.11 c	113.44 c	
	60	CE-8	60.48 e	83.83 d	44.80 c	15.79 d	28.61 ab	5.17 h	274.97 c	12.30 i	61.30 h	
	70	CE-9	85.40 bc	121.35 b	62.29 b	22.15 d	53.72 a	223.63 a	29.65 d	92.81 a	ND	
Factors			UE (µg/g FW)									
Solvent	Con (%)	Label	Cy-3-gal	Pn-3-glu	Mv-3-gal	Pn-3-ara	Mv-3-glu	Mv-3-ara				
Acetone	60	UE-1	124.40 a	43.34 bc	206.95 c	21.07 c	92.35 c	ND				
	70	UE-2	137.33 a	59.10 b	263.88 a	34.72 c	112.72 a	ND				
	80	UE-3	125.53 a	ND	235.97 b	27.32 c	100.34 b	ND				
Ethanol	60	UE-4	67.28 b	26.22 c	158.09 e	18.11 c	62.04 e	ND				
	70	UE-5	62.16 b	27.82 c	161.44 d	13.78 c	66.95 d	ND				
	80	UE-6	63.65 b	12.40 c	18.63 f	97.50 b	ND	ND				
Methanol	60	UE-7	ND	11.77 c	19.59 ef	278.42 a	12.28 g	ND				
	70	UE-8	81.94 b	320.65 a	15.43 g	114.27 b	ND	56.62 a				
	80	UE-9	63.11 b	256.16 a	4.28 h	18.80 c	56.16 f	ND				
[B] Cherry							[C] Red pear peel					
Factors			CE (µg/g FW)		Factors		UE (µg/g FW)		Factors		CE (µg/g FW)	
Solvent	Temp (°C)	Label	Cy-3-rut		Solvent	Con (%)	Label	Cy-3-glu	Solvent	Temp (°C)	Label	Cy-3-gal
Acetone	50	CE-1	1.49 g		Acetone	60	UE-1	3.50 e	Acetone	50	CE-1	4.09 bcd
	60	CE-2	4.96 ef			70	UE-2	7.79 c		60	CE-2	6.60 ab
	70	CE-3	6.00 de			80	UE-3	7.32 cd		70	CE-3	5.50 bcd
Ethanol	50	CE-4	4.14 f		Ethanol	60	UE-4	14.98 a	Ethanol	50	CE-4	8.57 a
	60	CE-5	9.92 b			70	UE-5	11.70 b		60	CE-5	6.39 abc
	70	CE-6	7.91 c			80	UE-6	11.39 b		70	CE-6	3.95 cd
Methanol	50	CE-7	6.60 d		Methanol	60	UE-7	15.53 a	Methanol	50	CE-7	4.31 bcd
	60	CE-8	12.06 a			70	UE-8	14.59 a		60	CE-8	3.41 d
	70	CE-9	9.26 b			80	UE-9	10.17 bc		70	CE-9	4.04 cd

* Columns with *post-hoc* LSD test (*) were only illustrated for fruits and treatment factors showing the significant effect in ANOVA results (shown in Table 3); Means followed by the same letter within each extraction method and the type of fruits were not significantly different ($P>0.05$) based on LSD test.

[A] Total phenolic content

[B] Total monomeric anthocyanin

[C] DPPH radical scavenging capacity

[D] Percent polymeric color

Fig. 1. The effect of main treatment factor on total phenolic content (TPC) [A], total monomeric anthocyanin (TMA) [B], DPPH radical scavenging activity [C], and percent polymeric color (%) [D] of three types of fruit extracts using either conventional solvent extraction (CE) or ultrasound-assisted extraction (UE) based on analysis of variance (ANOVA) and multiple comparison *post-hoc* least significant difference (LSD); **This figure only reported the results on the fruit and treatment factors that showed significant treatment effect**; The same lowercase letter above each bar within each extraction method and the type of fruits were not significantly different ($P>0.05$) based on LSD test.

Fig. 2. Anthocyanin composition profiles in blueberry, cherry, and red pear peel extracts analyzed by HPLC; Blueberries contain (1) delphinidin-3-galactoside, (2) delphinidin-3-glucoside, (3) cyanidin-3-galactoside, (4) delphinidin-3-arabinoside, (5) cyanidin-3-glucoside, (6) petunidin-3-galactoside, (7) cyanidin-3-arabinoside, (8) petunidin-3-glucoside, (9) petunidin-3-arabinoside, (10) peonidin-3-glucoside, (11) malvidin-3-galactoside, (12) peonidin-3-arabinoside, (13) malvidin-3-glucoside, and (14) malvidin-3-arabinosides; Cherries mainly contain (1) cyanidin-3-glucoside, (2) cyanidin-3-rutinoside, and (3) unknown; Red pear peels mainly contain (1) cyanidin-3-galactoside, and (2) cyanidin-3-glucoside.

Fig. 3. PCA for blueberry extraction conditions on individual anthocyanins.

Delphinidin-3-galactoside = Dp-3-gal; Delphinidin-3-glucoside = Dp-3-glu; Cyanidin-3-galactoside = Cy-3-gal; Delphinidin-3-arabinoside = Dp-3-ara; Cyanidin-3-glucoside = Cy-3-glu; Petunidin-3-galactoside = Pt-3-gal; Cyanidin-3-arabinoside = Cy-3-ara; Petunidin-3-glucoside = Pt-3-glu; Petunidin-3-arabinoside = Pt-3-ara; Peonidin-3-glucoside = Pn-3-glu; Malvidin-3-galactoside = Mv-3-gal; Peonidin-3-arabinoside = Pn-3-ara; Malvidin-3-glucoside = Mv-3-glu; Malvidin-3-arabinosides = Mv-3-ara.

*The specific extraction conditions of blueberry label as CE-1 to CE-9 and UE-1 to UE-9 could be found in Table 4.