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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.

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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 flavylium), 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 (Ignat, Volf, & Popa, 2011; Wrolstad et al., 2005). 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%

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for extracting complex structures of anthocyanins with different polarities (Deng, Penner, & Zhao, 2011). Turkmen, Sari, and 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 (Pinelo et al., 2007; Spigno & De Faveri, 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 (Virot, 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 (Bai, Zhang, & Ren, 2013; Chen et al., 2007; Yue, Shao, Yuan, Wang, & Qiang, 2012). 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

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