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Isolation of high-purity anthocyanin mixtures and monomers from blueberries using combined chromatographic techniques

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ABSTRACT

Research on the isolation and preparation of anthocyanins has intensified in recent years because of the requirements of quantitative and bioactive analyses. However, simple and effective methods for the scale purification of pure anthocyanins from natural products are rarely reported. In this study, high-purity anthocyanin mixtures and monomers were successfully isolated from wild blueberries using a combination of column chromatography and semi-preparative HPLC. We established an effective elution system to separate high-purity anthocyanin mixtures with aqueous ethanol containing 0.01% HCl first in an Amberlite XAD-7HP column (ethanol/H_2O = 35:65) and then in a Sephadex LH-20 column (ethanol/H_2O = 25:75). Crude anthocyanin extracts were isolated using the Amberlite column, and a purity of 32% was obtained based on UV–vis analysis. Three fractions of anthocyanin mixtures were isolated from the crude extracts using the Sephadex column with purities ranging from 59% to 68%. Three pure monomeric anthocyanins of malvidin-3-O-glucoside, petunidin-3-O-glucoside, and delphinidin-3-O-glucoside were also isolated by semi-preparative HPLC and identified by HPLC-DAD-ESI–MS/MS. The purities of these anthocyanins were determined by analytical HPLC and estimated to be 97.7%, 99.3%, and 95.4%, respectively. The results of this study may help promote the purification of anthocyanins from most blueberry varieties as well as from other plant materials.

1. Introduction

Anthocyanins are naturally occurring phenolic secondary metabolites that belong to the flavonoid family. As water-soluble natural pigments, anthocyanins are promising alternatives to synthetic food colorings [1]. Besides their colorant properties, increasing evidence shows that anthocyanins exhibit potential health benefits [2–4]. Unfortunately, at present, low extraction percentages, instability, and difficulties in obtaining expensive standards hamper further bioactivity research on anthocyanins. As such, most of the research on anthocyanins is limited to the use of crude anthocyanin extracts from vegetables or fruits. However, the presence of non-anthocyanin phenolic compounds and other impurities inevitably interferes with the evaluation of the biological activities of crude anthocyanin extracts [1,5]. Considering these issues, isolation and preparation of pure anthocyanin standards from plant sources are needed for accurate quantification purposes.

Extensive research suggests that wild blueberries have a higher antioxidant capacity than cranberries, strawberries, plums, raspberries, and cultivated blueberries [6]. Wild blueberries have also been reported to be beneficial in maintaining memory function [7], inhibiting cancer growth [8], preventing atherosclerosis [9], and promoting gastrointestinal and digestive health [10]. Anthocyanins contribute to the beneficial properties of wild blueberries [7,11,12]. Despite their significant potential value, however, wild blueberries have not been widely used by the food industry because they are often consumed directly or used to produce juices and fruit wines. High value-added anthocyanin products (e.g., high-purity anthocyanin extracts) are still unavailable in the market. Thus, while technologically challenging, the preparation of pure anthocyanins from wild blueberries is a very promising endeavor [13,14].

The separation of anthocyanins from plant materials has been carefully studied using techniques such as solid-phase extraction (SPE) [15], high-speed counter-current chromatography (HSCCC) [16], column chromatography (CC) [17,18], and preparative high-performance liquid chromatography (HPLC) [19,20]. Despite the popularity of SPE and HSCCC, the residuals of immiscible organic solvents obtained via these techniques are difficult to remove, which is detrimental to further bioactivity experiments.

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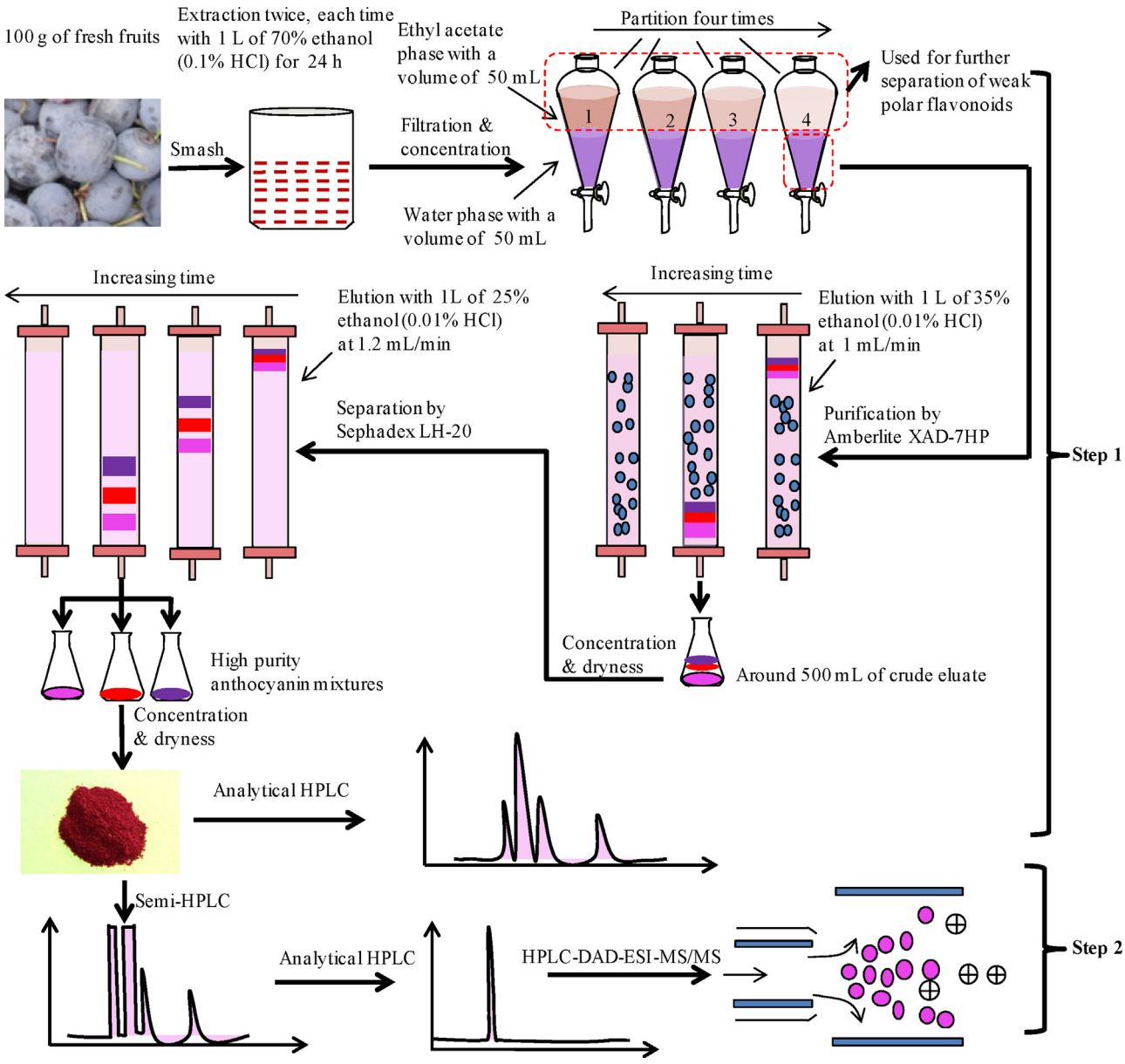

Fig. 1. An effective process for the preparation of high-purity anthocyanin mixtures (step 1) and anthocyanin monomers (step 2) from wild blueberry fruits.

As an important purification technology, CC has been widely used to isolate flavonoids, tannins, and monomeric anthocyanins, especially with the extensive application of Amberlite XAD-7 and Sephadex LH-20 columns [17,21,22]. However, most of the extraction procedures developed thus far involve toxic organic solvents, including methanol, acetone, formic acid, acetic acid, trifluoroacetic acid (TFA), and acetonitrile. Although the anthocyanin content of Chinese wild blueberries has been previously determined [23,24], data on the feasibility and systematic isolation of high-purity anthocyanins are insufficient. The present study was conducted in response to recent research issues concerning the nutritional and health benefits of blueberries, especially the wild blueberry variety. The aims of this study are: (1) to develop a natural and low-cost method for the purification of anthocyanin mixtures using combined CC techniques and (2) to explore a semi-preparative HPLC technology for obtaining pure monomeric anthocyanins from Chinese wild blueberry fruits.

2. Materials and methods

2.1. Reagents and standards

HPLC-grade Formic acid and methanol were purchased from Merk (Darmstadt, Germany). Ethanol, ethyl acetate (EtOAc) and hydrochloric acid (HCl) of analytical grade were purchased from Beijing Chemistry Factory (Beijing, China). Deionised water was obtained from a Milli-Q Element water purification system (Millipore Co., Billerica, MA, USA). Standard of cyanidin-3-O-glucoside was purchased from Sigma–Aldrich Chemical Co. (St Louis, MO, USA).

2.2. Plant materials

Fresh, ripe samples of lowbush wild blueberries fruits of the *Vaccinium uliginosum* L. species were collected from unmanaged,