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Separation and identification of polyphenols in apple pomace by high-speed counter-current chromatography and high-performance liquid chromatography coupled with mass spectrometry

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ABSTRACT

Apple pomace, a by-product in the processing of apple juice, was investigated as a potential source of polyphenols. Two methods of separation and purification of polyphenols from apple pomace extract were established by combination of gel chromatography with high-speed counter-current chromatography (HSCCC) and solvent extraction with HSCCC, respectively. The optimal separation was performed on a Sephadex LH-20 column using gradient aqueous ethanol as eluting solvent from 0% to 100% in increments of 10%. HPLC analysis indicated that main polyphenols existed in fractions eluted between 40% and 50% aqueous ethanol. The fractions of interest from column were separated by HSCCC with the solvent system hexane–ethyl acetate–1% aqueous acetic acid (0.5:9.5:10, v/v/v). Ethyl acetate fractionation of the apple pomace extract followed by direct HSCCC separation by the same solvent system in the volume ratio of 1:9:10 also produced a good separation of the main polyphenols of interest. Six high-purity polyphenols were achieved tentatively and identified by HPLC/MS: chlorogenic acid (1, *m/z* 354), quercetin-3-glucoside/quercetin-3-glacaside (2, *m/z* 464), quercetin-3-xyloside (3, *m/z* 434), phloridzin (4, *m/z* 436), quercetin-3-arabinoside (5, *m/z* 434), and quercetin-3-rhamnoside (6, *m/z* 448). These results provided a preliminary foundation for further development and exploration of apple pomace.

1. Introduction

Apple pomace is a by-product in apple juice production, representing around 30% of the original fruit, consisting of peel, core, seed, calyx, stem, and soft tissue. A large quantity of apple pomace is produced worldwide every year and its disposal has caused a serious environmental problem. Researchers have proposed the use of apple pomace for the production of different value-added products including enzymes, organic acids, ethanol, aroma compounds, and natural antioxidants [1].

As is well known, apples represent an important source of bioavailable polyphenolic compounds such as flavonols, monomeric and oligomeric flavanols, dihydrochalcones, and anthocyanidins [2]. The contents of phenolic compounds vary greatly among different varieties and different parts of apples, and apple peels contain a higher concentration of phenolic compounds than the flesh [3]. Conventional apple juice production results in a juice poor in phenolic compounds and with only 3–10% of the antioxidant activity of the fruit they are produced from [4], most of the compounds remaining in the apple pomace. As a result of its abundance, and owing to the increasing interests in new natural sources of antioxidant products, apple pomace has been investigated as a potential source of bioactive polyphenols during recent years [5–8], which can be used for various purposes in the food, pharmaceutical and cosmetic industry for their effective antioxidant and free radical scavenger activities.

The separation of polyphenolic compounds in apple pomace involved repetitive Sephadex LH-20 chromatography [6,7]. High-speed counter-current chromatography (HSCCC) has been applied for the separation and fractionation of procyanidins from apples using two-phase solvent system composed of *n*-butanol–methyl *tert*-butyl ether–acetonitrile–0.1% trifluoroacetic acid (2:4:3:8) [9] and methyl acetate–water (1:1) [10]. Size-exclusion chromatography and normal-phase, high-performance liquid chromatography were also employed for the fractionation of apple procyanidins according to their degree of polymerization [11,12].

In this paper, two methods of separation and purification of polyphenols from apple pomace extract were established by combination of Sephadex LH-20 chromatography with HSCCC, and solvent extraction with HSCCC, respectively. The derived compounds were identified by HPLC/MS.

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2. Experimental

2.1. Apparatus

The present study employed two different HSCCC units, a model GS20 analytical and a model GS10A3 preparative HSCCC system manufactured by the Beijing Institute of New Technology Application, Beijing, China. The multilayer coil of the analytical unit was prepared by winding 0.8 mm I.D. (1.6 mm I.D. for preparative unit) PTFE tubing coaxially onto a spool-shaped column holder. The beta value ranged from 0.4 to 0.72 (0.5–0.75 for preparative unit), and the total capacity was 35 mL (220 mL for preparative unit).

The HPLC/MS analyses were performed on an Agilent 1100 HPLC system coupled with diode array detection (DAD) and an Agilent 1100 series MSD trap (SL model) with an electrospray ionization (ESI) interface. The HPLC system is also equipped with a quaternary pump and an autosampler.

2.2. Reagents and materials

A glass column (60 cm × 2.5 cm I.D.) was provided by Beijing Glass Apparatus Company (Beijing, China). 80 g Sephadex LH-20, analytical grade, provided by Amersham Biosciences (Shanghai, China), was packed into the column. The bed volume was about 250 mL.

All organic solvents used for crude extraction, column chromatography, and HSCCC separation were of analytical grade and provided by Beijing Chemical Reagents Co. (Beijing, China); methanol of HPLC grade was from Dikma Scientific, USA.

2.3. Extraction of apple pomace

A 3-kg amount of dry and ground apple pomace powder was soaked in 5 L of ethanol overnight. The residual was extracted for two more times. Then the solution were combined, concentrated and dried in a vacuum oven at 40 °C. A 116-g amount of crude extract was obtained.

2.4. Pretreatment of the apple pomace crude extract

2.4.1. Pre-separation of polyphenols through Sephadex LH-20 column

A 10-g amount of ethanol extract of apple pomace was fractioned through a Sephadex LH-20 column, eluted with aqueous

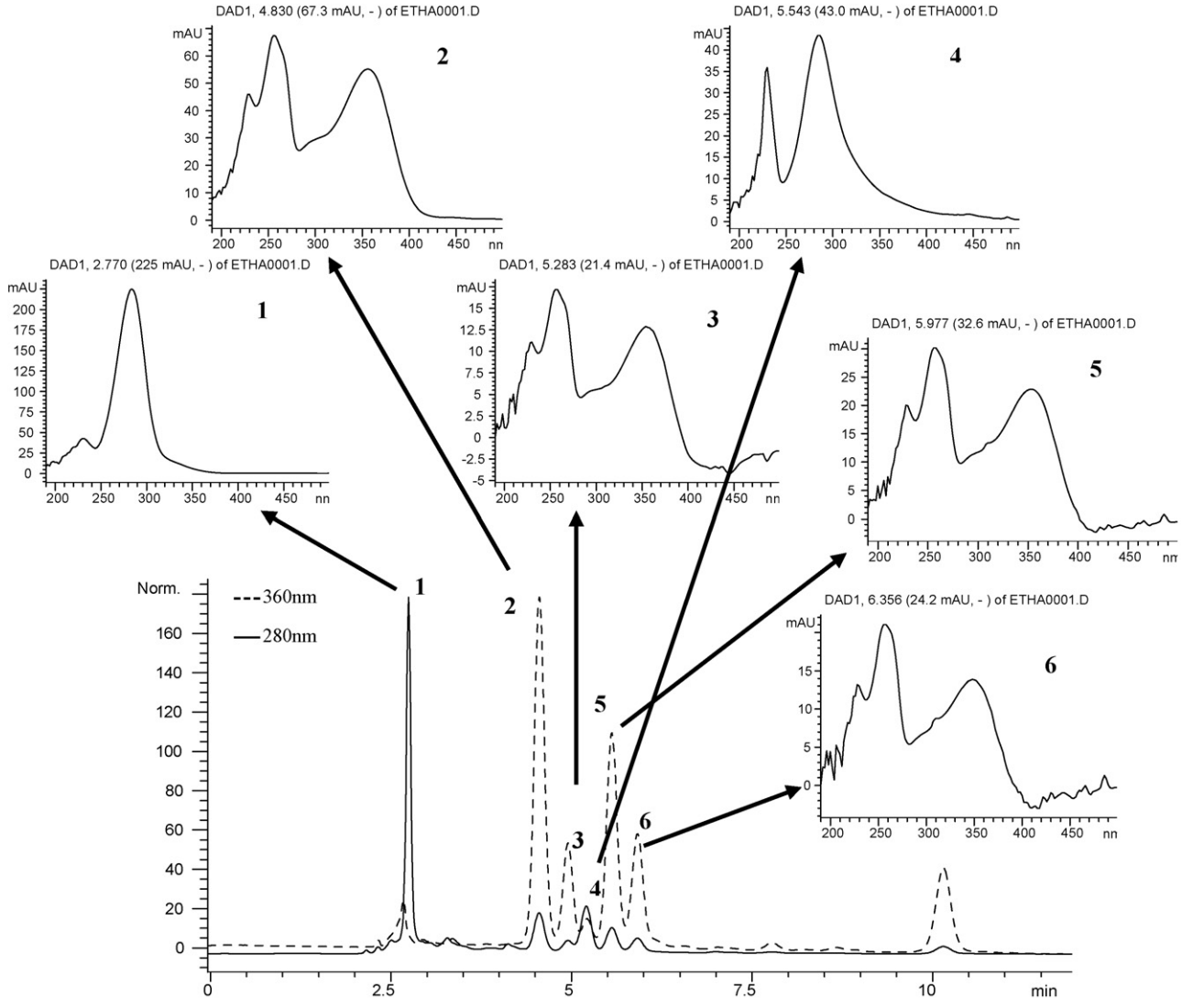

Fig. 1. HPLC profile of the ethanol extract of apple pomace and the UV spectrum of each peak. Conditions: column: Zorbax SB-C_{18} (250 mm × 4.6 mm I.D., 5 μm); mobile phase: methanol–water with 2% acetic acid (1:1, v/v). Detection: 280, 360 nm; flow-rate: 1 mL/min.