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RESEARCH ARTICLE

Recovery of Arbutin in High Purity from Fruit Peels of Pear (*Pyrus pyrifolia* Nakai)

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Abstract β-Arbutin is one of the most abundant compounds in pear fruit and has been widely used as a whitening agent in cosmetic products. In this study, a simple method for the purification and isolation of arbutin in high purity from the fruit peel of a pear (*Pyrus pyrifolia* Nakai cv. Chuhwangbae) was developed. The high extraction efficiency of arbutin was accomplished with 80% ethanol under acidic conditions (pH 3.0). In the partition between water and ethyl acetate, almost all of the arbutin (78%) was detected in the water layer. Therefore, arbutin was further purified and isolated from the water layer using Diaion HP-20 column chromatography and preparative HPLC. The isolated arbutin represented a 60% recovery with a purity exceeding 99%.

Keywords: pear, *Pyrus pyrifolia*, fruit peel, arbutin, Diaion HP-20 column chromatograpy

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Introduction

Arbutin (4-hydroxyphenyl glucopyranoside), which is a tyrosinase inhibitor, has been widely used as a whitening agent in cosmetic products (1). There are 2 anomeric forms of arbutin; α- and β-arbutins (Fig. 1). Both arbutins have been reported to inhibit tyrosinase activity in mushroom and mouse melanoma (2). Of these 2 arbutins, α-arbutin (4-hydroxyphenyl α-D-glucopyranoside) showed a stronger inhibitory activity than β-arbutin (4-hydroxyphenyl β-D-glucopyranoside) against mammailian and mouse melanoma tyrosinases (3,4). α-Arbutin glycosides were enzymatically synthesized from hydroquinone and sugars (2-5). However, it is very difficult to manufacture the α-arbutin glycosides by synthesis and people in general have issues with synthetic compounds. β-Arbutin is naturally found in various plants such as *Arctostaphylos uva-ursi* (Ericaceae), Rosaceae, and *Bergenia crassifolia* (Saxifragaceae) (6-9). These plants have been commonly used for the treatment of urinary problems such as urinary tract infection, cystitis, kidney stones, and diuretic (10).

Pears (*Pyrus* spp.) are widely distributed throughout the world, especially in Europe and Asia. Asian pears have been cultivated mainly in Eastern Asia including Korea, China, and Japan. The major species of Asian pears are *Pyrus bretschnrideri*, *P. pyrifolia* Nakai, *P. ussuriensis* Maxim., and *P. sinkiangensis* Yu (11). The fruit is usually consumed fresh and in processed foods such as juice, jellies, and jams. The fruit peels are produced in large quantities as byproducts during the manufacturing of pear products. Recently, Ramadan and Morsel (12) developed a method to recover lipids such as polyunsaturated fatty acids, tocopherols, and sterols from the fruit peels of pears. β-Arbutin is one of the phenolic compounds found in pear fruits along with chlorogenic acid and rutin (13,14). In

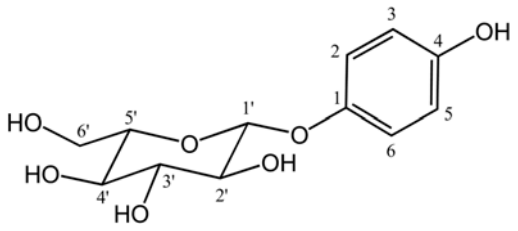

Fig. 1. Structure of β-arbutin (4-hydroxyphenyl β-D-glucopyranoside).

addition, β-arbutin has also been used as a specific marker to evaluate the authenticity of pear products (15-17). Fresh fruit peals were reported to contain an arbutin content of approximately 0.1%, although this value varies depending on the cultivars and ripening of fruit (11). Based on these finding, fruit peels have been suggested to be a good source of material for purification of arbutin, which is a natural whitening agent used in cosmetic products. However, studies on the purification of arbutin in high purity from pear and other plants have not yet been conducted. Therefore, in this study, a simple method for the extraction and purification of arbutin in high purity from the fruit peels of pear was developed using solvent fractionation, Diaion column chromatography, and the more readily available and economical method of HPLC.

Materials and Methods

Materials and chemicals The fresh fruits of pear, *Pyrus pyrifolia* Nakai cv. Chuhwangbae, grown on Naju city, South Korea, were harvested in September 2008 and identified by prof. Wol-Soo Kim, Laboratory of Pomology, College of Agriculture and Life Science, Chonnam National University. A voucher sample (No. JNU PE 20050831-2) was previously deposited in the herbarium of the laboratory. The fruits were hand-peeled with a peel thickness of about 3 mm and the peel fraction was stored at −70°C until used. The solvents used for analyses were of HPLC grade and purchased from Fisher Scientific Korea (Seoul, Korea). Spectrophotometric grade trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (EtOH) and ethyl acetate (EtOAc) used for extraction and solvent fractionation were of extra pure quality and were obtained from Duksan (Ansan, Korea). Authentic arbutin (4-hydroxyphenyl β-D-glucopyranoside, β-arbutin) was obtained from Sigma-Aldrich.

Extraction The fresh peels (10 g fresh wt.) were homogenized using a homogenizer (BM-2 Nissei bio-mixer; Nihonseiki, Osaka, Japan) in 20, 40, 60, 80, and 100% EtOH (200 mL) under acidic (pH 3.0, by 1 N HCl) and neutral conditions, respectively. After extraction for 24 h at room temperature, the extract was filtered under vacuum through No. 2 filter paper (Whatman, Maidstone, UK) and then the filtrate was concentrated by vacuum evaporation at 38°C. Each extract was dissolved in 1.0 mL of MeOH and then filtered through a polyvinylidene fluoride membrane (PVDF, Millex-HV 13 mm, 0.45 μm pore size, Millipore, Billerica, MA, USA) and subjected to HPLC analysis on an analytical scale.

Solvent fractionation The 80% EtOH (pH 3.0) extracts (1.35 g) of fruit peels (10 g fresh wt.) were suspended in 250 mL of water (H_2O) and partitioned with 250 mL of EtOAc (3 times). The EtOAc and H_2O layers were concentrated *in vacuo* at 38°C. These layers were dissolved in 1.0 mL of MeOH and then filtered through a PVDF membrane (Millex-HV 13 mm, 0.45 μm pore size, Millipore) and analyzed by HPLC on an analytical scale.

Isolation and purification of arbutin The H_2O layer obtained after the solvent fractionation of the 80% EtOH (pH 3.0) extract was chromatographed on a Diaion HP-20 column (3.5×45 cm, Mitsubishi Chemical Industrial, Tokyo, Japan). The elution was performed with EtOH/H_2O (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0, v/v, each 500 mL). The arbutin fraction was subjected to preparative HPLC (SPD-M20D; Shimadzu, Kyoto, Japan) equipped with a Shim pack prep-ODS(H)KIT column (20 mm i.d. ×250 mm, 10 μm, Shimadzu). Sample elution was detected at 280 nm using an HPLC-PDA system (SPD-M20D; Shimadzu) and flow rate of 9.9 mL/min. Sample elution was achieved by using a gradient of H_2O (pH 2.65 by TFA, eluent A) to 50% MeOH (eluent B), starting with 100% A, which was then increased to a mixture of 85% A and 15% B for 5 min. Finally the sample was eluted under isocratic conditions in the same mobile phase for 25 min.

Analysis of TLC Each fraction obtained from Diaion HP-20 column chromatography was spotted on a silica gel TLC plate (silica gel 60 F_{254}, 0.25 mm thickness; Merck, Darmstadt, Germany). The TLC plate was developed with BuOH/acetic acid/H_2O (4:1:1, v/v/v). After drying, the TLC plate was stained with iodine.

HPLC analysis of arbutin in analytical scale HPLC analysis for the analytical characterization of the intermediate and final products was carried out using a Chromeleon™ system (Dionex, Sunnyvale, CA, USA) equipped with a P580 pump, ASI-100 automated sample injector, and UVD-170S UV/VIS detector. Arbutin were separated on a ODS-80Ts column (4.6 mm i.d. ×250 mm, 5 μm, Tosoh, Kyoto, Japan). The mobile phase was composed of H_2O/acetic acid (98:2, v/v, eluent C) and MeOH/H_2O (60:40,