Separation and Purification Technology 170 (2016) 294–305

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

A new approach for obtaining *trans*-resveratrol from tree peony seed oil extracted residues using ionic liquid-based enzymatic hydrolysis *in situ* extraction

Fengli Chen [a,b], Xinglong Zhang [c], Xinqi Du [a,b], Lei Yang [a,b,*], Yuangang Zu [a,b], Fengjian Yang [a,b]

[a] Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, 150040 Harbin, Heilongjiang, China
[b] State Engineering Laboratory for Bioresource Eco-Utilization, Northeast Forestry University, 150040 Harbin, Heilongjiang, China
[c] Ganzhou Forest Industry Bureau, 341000 Ganzhou, Jiangxi, China

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 26 June 2016
Accepted 29 June 2016
Available online 30 June 2016

Keywords:
Enzymatic hydrolysis *in situ* extraction
trans-Resveratrol
Polydatin
Enzyme
Ionic liquid
Tree peony seed oil extracted residues

ABSTRACT

A new approach for ionic liquid-based enzymatic hydrolysis *in situ trans*-resveratrol extraction (ILEHE) from tree peony seed oil extracted residues is presented, in which enzymatic hydrolysis is used in an ionic liquid aqueous medium to enhance the *trans*-resveratrol yield. Various factors of the ILEHE procedure, including the variety and concentration of enzyme, type and concentration of ionic liquid, liquid–solid ratio and pH of the solution system, and ILEHE temperature and time, were investigated by single-factor experiments, response surface methodology, and first-order kinetic models. A satisfactory yield of *trans*-resveratrol (5.48 ± 0.14 μmol/g) was obtained by the novel developed approach compared with other conventional techniques. Scanning electronic microscopy of the samples indicated that tree peony seed treated by mixed cellulase and pectinase (1/1, w/w) enzymes in an ionic liquid led to more efficient extraction by reducing mass transfer barrier. The proposed ILEHE method was validated by stability, repeatability, and recovery experiments and shows promising prospects in the extraction and hydrolysis aspects, for enhancing efficiency of target components in natural products.

1. Introduction

trans-Resveratrol, a phytoalexin, is usually synthesized by an organism under conditions of stress, such as infection, trauma [1], ultraviolet irradiation, and exposure to ozone [2]. In recent years, *trans*-resveratrol has been widely applied in the fields of medicine, food, and cosmetics because of its important biological characteristics, including antioxidant activity, anti-inflammatory activity [3], cardiac protection action [4], anticancer activity, and inhibition of platelet aggregation. These uses have attracted increasing interest in *trans*-resveratrol itself [5] and genetic engineering has been carried out to achieve a strain of *Saccharomyces cerevisiae* to produce *trans*-resveratrol [6–8]; however, highly professional and technical requirements have limited its application at an industrial scale. Solvent extraction is currently the main method for obtaining *trans*-resveratrol from plant materials [9–12]; however, the concentration of *trans*-resveratrol in plants is usually small, so it is difficult and expensive to obtain large quantities by large-scale procedures. Some *trans*-resveratrol glycosides, such as *trans*-resveratrol-3-*O*-β-glucoside (polydatin) or *trans*-resveratrol-4-*O*-β- glucoside, are in fact usually found to be present at much higher contents in plants than that of *trans*-resveratrol (Fig. 1).

The tree peony plays an irreplaceable role in the ornamental, medicinal, and food industries [13]. The antioxidant activity of tree peony has led to its wide application in medicine [14,15]. In recent years, the tree peony seed has attracted attention in the food industry [16,17] because it is a potential resource for edible oil that is rich in α-linolenic acid and has beneficial effects on human nutrition and health. Recognizing its nutritional functions, increasing demand for peony seed oil will produce large quantities of tree peony seed oil extracted residues as a byproduct that will be disposed of as landfill waste or used as a low-value fuel. It has been found that valuable *trans*-resveratrol and its glycosides exist in the peony seed [18–20]. Ignoring soluble intracellular substances, the structure of the tree peony seed cell wall is mainly composed of pectic polysaccharides and structural proteins, which can be degraded by enzyme technology. Enzymes are protein molecules and biological catalysts that have the ability to enhance reaction rates. The catalytic roles of enzymes are most readily performed in an aqueous phase because enzymes are water-soluble [21].

* Corresponding author at: Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, China.
E-mail address: ylchennefu@163.com (L. Yang).

http://dx.doi.org/10.1016/j.seppur.2016.06.056
1383-5866/

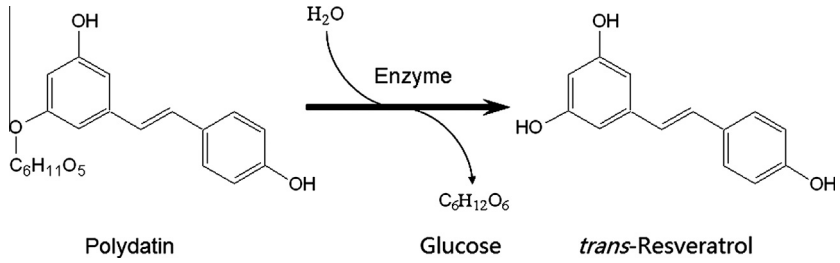

Fig. 1. Schematic of the transformation process of the *trans*-resveratrol glycoside, polydatin, to free *trans*-resveratrol.

The method of enzyme-assisted extraction has been recently applied in target compound extraction from traditional plant materials, and has shown the characteristics of flexibility, environmental compatibility, and high efficiency [22]. A series of enzymes, including cellulase, xylanase, dextranase, β-glucosidase, and pectinase, has been used and compared for their ability to hydrolyze cell wall constituents, disintegrate the cell wall frame, enhance dissolution of intracellular substances, and improve the yield of target compounds [23,24]. Aglycone *trans*-resveratrol is traditionally obtained by hydrolyzation of its glucosides, in which enzymatic catalysis is often used as the hydrolytic reagent: for example, cellulose and *β*-glucosidase were applied in *P. cuspidatum* for transforming polydatin to *trans*-resveratrol [25]. The usual steps for obtaining the lipophilic target component, *trans*-resveratrol, are as follows: (1) the plant material powder containing *trans*-resveratrol or its glycosides is suspended in deionized water containing an enzyme; (2) the mixture is incubated at appropriate conditions to hydrolyze the macromolecular components of the cell wall and *trans*-resveratrol glycosides; (3) the hydrolysis solution is removed from the suspension; (4) excess water is removed by freeze- or high-temperature-drying from the wet plant powder; (5) the plant powder is extracted by an appropriate concentration of organic solvent to obtain the lipophilic target component *trans*-resveratrol under appropriate conditions. The above processing scheme is tedious, complex, and requires high energy consumption, thus limiting its wide application. The development of simple, high-yielding, efficient, and environmentally friendly approaches, using new solvent catalysts for hydrolysis and *in situ* extraction of the target *trans*-resveratrol ingredient is therefore an important goal.

Ionic liquids are composed of an inorganic or organic anion and a bulky asymmetric cation. They have been used in different applications, including analysis, organic synthesis [26], as a reaction medium [27], and in separation science [28,29], because of their excellent chemical and physical properties that include good stability, tunable viscosity, negligible vapor pressure, wide liquid range, good miscibility in water and organic solvents, and good solubility and extractability for different organic compounds, and which are easily controlled compared with conventional solvents [30]. Ionic liquids therefore possess good properties as reaction solvents and have been successfully used in the separation of bioactive compounds from plant materials, including lignans [30], terpene lactones [31], quinones [32], alkaloids [33], and phenolic compounds [34]. Extensive investigations of enzymatic activity in ionic-liquid solutions have been performed [35], and have achieved beneficial effects. Liu et al. [36] recently obtained meaningful results using cellulose in an ionic-liquid solution to treat *Eucommia ulmoides* leaves to extract chlorogenic acid. To the best of our knowledge, however, there has been no research reported in the literature on the enzymatic hydrolysis of cell walls to improve penetration and simultaneous hydrolysis of *trans*-resveratrol glycosides to improve yields of *trans*-resveratrol, and use of ionic-liquid aqueous solution as a solvent to extract aglycone *trans*-resveratrol.

In the present paper, a novel extraction design, comprising ionic liquid-based enzymatic hydrolysis *in situ* *trans*-resveratrol extraction (ILEHE), is proposed for the improving the yield of *trans*-resveratrol from tree peony seed oil extracted residues. Various enzymes and ionic liquids were selected to compare the yields of *trans*-resveratrol from this source. The effects of changing the ionic liquid concentration, amount of enzyme, ILEHE temperature and time, liquid–solid ratio, and pH on the yield of *trans*-resveratrol were investigated using a factorial design, response surface methodology (RSM) with a Box–Behnken design (BBD), and first-order kinetic models. The ILEHE method proposed in this study is compared with traditional techniques, and validated in stability, repeatability, and recovery experiments. The microstructures of unprocessed and processed tree peony seed oil extracted residues were evaluated by scanning electron microscopy.

2. Experimental

2.1. Materials and chemicals

Paeonia rockii seeds were collected during September 2014 from the suburbs of Lanzhou (Gansu, China). Deshelling was performed using a shelling machine to obtain the kernels used for oil extraction. The kernels were dried at 80 °C for 8 h in an oven. The oil-extracted residue samples were obtained by the hexane extraction method used by Li et al. [37]. Before use, the samples were re-powdered by a disintegrator, sieved (60–80 mesh), and stored in closed desiccators at 4 °C. The samples moisture was determined as 7.6%. The same batch of samples was used for all experiments.

Reference *trans*-resveratrol and polydatin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and tetrahydrofuran of chromatographic grade were obtained from Merck (Darmstadt, Germany) and used for high-performance liquid chromatography (HPLC) analysis. The ionic liquids, 1-butyl-3-methylimidazolium bromide ([C_4mim]Br) and 1-butyl-3-methylimidazolium tetrafluoroborate ([C_4mim]BF_4), were purchased from Chengjie (Shanghai, China) and used without further purification. Cellulase (EC 1.1.1.27, ⩾400 U/mg), β-dextranase (EC 3.2.1.11, ⩾50 U/mg), xylanase (EC 3.2.1.8, 6000 U/mg), and pectinase (E.C.3.2.1.15, ⩾500 U/mg) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). All other reagents were of analytical grade and obtained from Aladdin (Shanghai, China). Deionized water, used for preparing and diluting all solutions, was purified by a Milli-Q water purification system (Millipore, Bedford, MA). Before HPLC analysis, all of the solutions were filtered through a 0.45-μm microporous membrane (Shanghai Yuanye Biotechnology Co., Ltd.) and degassed by ultrasonication.

2.2. High-performance liquid chromatography analysis and quantification

Standard stock solutions of polydatin (0.552 mg/mL) and *trans*-resveratrol (1.031 mg/mL) were prepared by dissolving the