Simultaneous Synergistic Microwave–Ultrasonic Extraction and Hydrolysis for Preparation of *trans*-Resveratrol in Tree Peony Seed Oil-Extracted Residues Using Imidazolium-Based Ionic Liquid

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Abstract

Imidazolium-based ionic liquid-based microwave–ultrasonic synergistic simultaneous extraction and hydrolysis (IMUSEH) method was developed to obtain *trans*-resveratrol from tree peony (*Paeonia rockii* (S.G.Haw & Lauener) T.Hong & J.J.Li) seed oil-extracted residues, in which the basic ionic liquid aqueous solution was used for extraction and transformation of *trans*-resveratrol glycosides to free *trans*-resveratrol. Through comparison, 1-butyl-3-methylimidazolium imidazolate ([C₄C₁im]Im) was selected as a dual solvent–catalyst, which showed an excellent solvent effect and catalytic activity. After optimization by a factorial design and response surface methodology, a higher yield of *trans*-resveratrol (3.06 ± 0.12 $\mu\text{mol/g}$) was obtained under optimal conditions (0.5 M [C₄C₁im]Im, 24 mL/g liquid–solid ratio, 25 min time, and 396 W microwave irradiation power). The developed technique was compared with ionic liquid-based microwave-assisted extraction and ionic liquid-based ultrasonic-assisted extraction based on first-order kinetic model and scanning electron microscopy, which highlighted the mechanism of the microwave–ultrasonic synergistic apparatus. Method validation studies showed that the proposed method can be used to prepare *trans*-resveratrol. The developed method showed promise in transforming glucoside to aglycone in plant materials.

Keywords: ionic liquid-based microwave–ultrasonic synergistic simultaneous extraction and hydrolysis; *trans*-resveratrol; tree peony seed; oil-extracted residues; basic ionic liquid; dual solvent–catalyst.

1. Introduction

In recent years, ionic liquids have been investigated extensively in different applications, such as reaction media (Melo et al., 2012), catalysts (Crosthwaite et al., 2008; Bogel-Lukasik et al., 2010), synthesis (Cooper et al., 2004) and separation science, because of the excellent properties (Faria and Bogel-Lukasik, 2015). The main application of ionic liquids was acting as reaction solvents in successful extraction and separation of different bioactive compounds from plant materials, including lignans (Prado et al., 2016), quinones (Liu et al., 2013), alkaloids (Lu et al., 2008) and flavonoids compounds (Chen et al., 2014). Although some disadvantages of ionic liquids are still existed, including high cost, complex preparation, and potential toxicity (Kulacki and Lamberti, 2008), resulting in ionic liquids cannot be applied in food extraction, ionic liquids are still regarded as efficient catalysts in some applications because of the good recoverability and negligible vapor pressure. Literatures indicate that the most popular application of acidic ionic liquid is in aglycone preparation. Some acidic ionic liquids, such as SO_3H -based ionic liquids, exhibit a catalytic effect in hydrolyzing the flavonoid glucosidic bond and the ester bond of hydrolysable tannins, to yield flavonols (Yao et al., 2012), ellagic acid, and gallic acid (Liu et al., 2016). Nevertheless, basic ionic liquids possess a higher catalytic ability and require mild conditions compared with acidic ionic liquids (Zhou et al., 2012; Ranu et al., 2007). The basic ionic liquid, 1-methyl-3-butylimidazolium hydroxide [Bmim]OH was first recommended by Ranu et al. as a strongly basic ionic liquid (Olah et al., 2005). It has been used as an efficient catalyst in different Brønsted alkali catalysis reactions, including Michael addition (Ranu et al., 2007), Knoevenagel condensation (Ranu and Jana, 2006), transesterification (Han et al., 2012) and carbohydrate dehydration (Qu et al., 2012). Therefore,

basic ionic liquids will be more effective than acidic ionic liquids in glycone production under moderate conditions. Recently, basic ionic liquids have been applied widely in organic reactions as a basic catalyst (Chen et al., 2008; Fan et al., 2013). In the researches, Meindelsma et al. (Meindelsma et al., 2005) pointed out that ionic liquids can separate aromatic hydrocarbons from mixtures of aromatic and aliphatic hydrocarbons, and Sun and Shi (Sun and Shi, 2012) indicated that basic ionic liquids of the imidazole anion can separate acidic compounds. Basic ionic liquids have been used as the extraction solvent in separation processes (Sun and Shi, 2012; Zawadzki et al., 2013).

The tree peony is prized for its beautiful ornamental flowers, and many cultivars of tree peony have been bred over thousands of years, for example, Ziban (*Paeonia rockii* (S.G.Haw & Lauener) T.Hong & J.J.Li). Tree peony has also been applied in medicine because of its pharmacological action, antioxidant activity (Li et al., 2009; Fan et al., 2012). Tree peony seed oil is rich in α -linolenic acid, which has beneficial effects on human nutrition and health. Ziban seed oil was approved as a new resource food by the Chinese Government in 2011. The market demand for peony seed oil is growing because of its nutritive function, which has resulted in the disposal of byproducts of large quantities of tree peony seed oil-extracted residues. These are disposed as landfill waste or are used as low-value fuel. In recent years, the use of tree peony seed oil-extracted residues has attracted much attention, which was beneficial to resources reuse and waste minimization. Valuable *trans*-resveratrol and its glycosides occur in tree peony seeds (Kim et al., 2002; He et al., 2010).

trans-Resveratrol, 3,5,4'-trihydroxy-*trans*-stilbene, a stilbenoid compound, is known for its

therapeutic effects and pharmacological activity such as antioxidant (Zhang et al., 2014a), anti-inflammatory (Udenigwe et al., 2008), anti-tumoral (Wang et al., 2014), cardiovascular protection (Elíes et al., 2013), anti-aging (Hsu et al., 2014), and anticancer (Kundu and Surh, 2008) activity. *trans*-Resveratrol is attracting scientific attention currently because of its ability to promote longevity, and it is being used extensively in medicine, food, and the cosmetic industries. The main way to obtain *trans*-resveratrol is by chemical synthesis, which is expensive and environmentally unfriendly (Lara-Ochoa et al., 2015; Farina et al., 2006). *trans*-Resveratrol has also been produced by a strain of *Saccharomyces cerevisiae* that is obtained by genetic engineering (Wang et al., 2011; Wang and Yu, 2011). Professional and technical requirements have limited its application at an industrial scale. *trans*-Resveratrol has therefore been obtained mainly by solvent extraction from plants, and a green extraction of *trans*-resveratrol technique has been studied by Mantegna et al. (2012). Its small concentration in plants makes obtaining large quantities of *trans*-resveratrol using large-scale extraction difficult and expensive. *trans*-Resveratrol glycosides, such as *trans*-resveratrol-3-O- β -glucoside (polydatin) or *trans*-resveratrol-4-O- β -glucoside, are usually present in higher concentrations than *trans*-resveratrol.

Traditionally, aglycone is achieved by glucoside hydrolyzation, in which the common hydrolytic reagent is inorganic acid or alkaline. However, the hydrolytic reaction requires aggressive polluting conditions (Liu et al., 2010; Wang et al., 2007). Enzymatic catalysis or microbial fermentation has been applied in the biotransformation of polydatin to *trans*-resveratrol in *Polygonum cuspidatum*. Cellulose (β -glucosidase) and immobilized β -glucosidase have been used for enzymatic catalysis (Wang et al., 2007; Zhang et al., 2014b),

but its industrial development is limited because of the high cost of enzymes. Another type of biotransformation method, including microbial fermentation and immobilized microbial fermentation (Wang et al., 2007; Jin et al., 2013), has been carried out under mild conditions with lower cost and less pollution, but toxic compounds were produced and a longer time (12–48 h) was required (Liu et al., 2010; Wang et al., 2007; Zhang et al., 2014b; Jin et al., 2013), which leads to a low efficiency. After transformation, the extraction process needs a large amount of anhydrous ethanol to maintain a suitable ethanol concentration because of the presence of water in the transformation system; this increases the use of organic solvent. The development of simple, high-yielding, efficient, and environmentally friendly approaches using new dual solvent–catalysts to hydrolyze *trans*-resveratrol glycosides is important.

Microwave- and ultrasonic-assisted extraction methods can enhance reaction and extraction efficiencies, shorten reaction and extraction times, and reduce energy consumption compared with conventional techniques. These important novel techniques have been developed extensively in separation science and organic synthesis in recent years (Hou et al., 2016; Chen et al., 2016; Zenevicz et al., 2016). Microwaves are high-frequency electromagnetic waves that enable the solute or solvent molecules to achieve instantaneous polarization, and stimulate a molecular-active part (polarity) to better contact and react. Microwave irradiation can be converted into heat energy rapidly to increase the internal cell temperature rapidly and to produce pressure on and rupture cell walls. This leads to components leaching into the surrounding solvent (Wang and Weller, 2006; Chen et al., 2015). Microwave energy can be absorbed and transferred efficiently by ionic liquid. Ultrasound waves can cause cavitation between solvent and samples, and the pressure produced by the cavitation can disrupt cell

walls, and increase permeability, which enhances the mass transfer of cell contents from the solid to the liquid phase (Chemat et al., 2011). Therefore, microwave–ultrasonic synergy apparatus that combined of microwave and ultrasound has been successfully applied in several extraction processes (Cravotto et al., 2008; Cravotto and Cintas, 2007), which is also promising for the preparation of *trans*-resveratrol. In this proposed process, basic ionic liquid aqueous solution was used as a dual solvent–catalyst. The role of basic ionic liquid in this process includes not only the ability of fast absorption of microwave for rapid heating of raw materials, effective dissolution ability of target analytes, but also the transformation ability of *trans*-resveratrol glycosides to free *trans*-resveratrol.

In this study, a basic imidazolium-based ionic liquid-based microwave–ultrasonic synergistic simultaneous extraction and hydrolysis (IMUSEH) method was developed to obtain *trans*-resveratrol from tree peony seed oil-extracted residues. Five types of 1-alkyl-3-methylimidazolium imidazolate were selected as the dual solvent–catalyst and compared with acetate radical and hydroxide radical anions. Operating parameters, including ionic liquid concentration, microwave irradiation power, IMUSEH time, and liquid–solid ratio were optimized using a factorial design and a response surface methodology (RSM). First-order kinetic models of the developed method, ionic liquid-based microwave-assisted extraction (IL-MAE) and ionic liquid-based ultrasound-assisted extraction (IL-UAE) were applied to highlight the mechanism of the microwave–ultrasonic synergistic instrument. Microstructures and chemical structures of the plant samples before and after treatment were investigated by scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR).

2. Experimental

2.1 Materials and Chemicals

2.1.1 Materials

Paeonia rockii seeds were from the Gansu Zhongchuan Peony Industry Co., Ltd (Lanzhou, China). Deshelling was performed using a shelling machine to obtain kernels. The kernels were oven dried for 6 h at 80 °C. The oil-extracted residue samples were obtained after *n*-hexane extraction as described by Li et al. (Li et al., 2015). Before use, samples were re-powdered using a disintegrator, sieved (60–80 mesh), and stored in closed desiccators at 4 °C. The sample moisture content was 9.6%. The same batch of samples was used throughout the experiments.

2.1.2 Chemicals

Reference *trans*-resveratrol and polydatin were from Sigma-Aldrich Inc. (St. Louis, MO, USA). Methanol for high-pressure liquid chromatography (HPLC) analysis was of chromatographic grade and was from J&K Chemical Ltd (Beijing, China). Ionic liquids, 1-ethyl-3-methylimidazolium imidazolid ([C₂C₁im]Im), 1-butyl-3-methylimidazolium imidazolid ([C₄C₁im]Im), 1-hexyl-3-methylimidazolium imidazolid ([C₆C₁im]Im), 1-octyl-3-methylimidazolium imidazolid ([C₈C₁im]Im), and 1-decyl-3-methylimidazolium imidazolid ([C₁₀C₁im]Im), reference-basic ionic liquid 1-butyl-3-methylimidazolium hydroxide ([C₄C₁im]OH), and 1-butyl-3-methylimidazolium acetate ([C₄C₁im]OAc) were from Shanghai Chengjie Chemical Co. Ltd (Shanghai, China) and were used without further purification

(water content and purity of all the ionic liquids was < 10000 mg/kg and $\geq 99\%$, respectively), the structures and properties of these basic ionic liquids are listed in Table 1. All other chemicals of analytical grade were from Beijing Chemical Reagents Co. (Beijing, China). Deionized water as purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for preparing and diluting solutions. Before HPLC analysis, all solutions were filtered through a $0.45\text{-}\mu\text{m}$ microporous membrane (Guangfu Fine Chemicals Research Institute, Tianjin, China) and were degassed by ultrasonication.

2.2 Apparatus

A microwave–ultrasonic synergistic apparatus (CW-2000; Shanghai Xintuo Analytical Instrument Technology Co., Shanghai, China) that consisted of a microwave oven with a continuous adjustable maximum power of 800 W and an ultrasonic transducer with a fixed power of 50 W was applied in the IMUSEH process. This system was equipped with a reflux condenser and a contact digital thermocouple continuous feedback temperature sensor to provide temperature control in the process. The bottom of flat-bottomed flask is glued with metal block through conductive adhesive, which has a good contact with the ultrasound transducer. The schematic diagram of the microwave-ultrasonic synergistic apparatus is presented in Figure 1.

The chromatographic system (Waters, Milford, MA, USA) consisted of Millennium 32 software, a 717 plus autosampler, 1525 pump, 717 automatic column temperature control box, and 2487 ultraviolet detector. The chromatographic separation of target analytes was

performed on a Kromasil-C18 reversed-phase column (AkzoNobel Pulp and Performance Chemicals AB Separation Products, Bohus, Sweden; 4.6 mm × 250 mm, 5 µm).

2.3. HPLC analysis and quantification

Standard stock solutions of *trans*-resveratrol (1.03 mg/mL) and polydatin (0.55 mg/mL) were prepared by dissolution in methanol. Before HPLC analysis, the standard solutions were stored at 4 °C in the dark, and were diluted with methanol to the required concentration.

The mobile phase used to separate target analytes was methanol:water:phosphoric acid (45:54.6:0.4, v/v/v), which was adjusted appropriately based on the reference (Zupančič et al., 2015). The detection conditions for HPLC were 1.0 mL/min mobile phase flow rate, 10 µL injection volume, 25 °C column temperature, 25 min running time, and 306 nm wavelength to detect *trans*-resveratrol and polydatin. The standard and sample solutions were filtered through 0.45-µm membranes before HPLC analysis. Under these conditions, polydatin and *trans*-resveratrol were separated at 7.26 and 16.82 min, respectively, which can be seen in Figure 2. Analyte identification was completed by comparing the retention time of chromatographic peaks with reference standards.

2.4. IMUSEH

Accurately weighed oil-extracted residues (0.5 g) were placed into flat-bottomed flasks that contained various types and volumes of ionic liquid aqueous solutions. Suspensions were placed in the microwave–ultrasonic synergistic apparatus and treated for a certain time at different power settings. After IMUSEH, the sample solution achieved each time was cooled

to room temperature rapidly and centrifuged at 5000 rpm for 15 min. The supernatant volume was measured, and 1 mL solution was filtered through a 0.45- μ m membrane prior to HPLC analysis. The cation and anion of the ionic liquids, ionic liquid concentration, liquid–solid ratio, microwave irradiation power, and IMUSEH time were optimized to obtain the best *trans*-resveratrol yield. Target analyte yield was calculated from:

$$Y = \frac{C \times V}{m \times M \times (1 - \varphi)} \times 1000, \quad (1)$$

where C is the mean concentration of *trans*-resveratrol or polydatin in the sample as determined by HPLC analysis for the three samples, mg/mL; V is the measured solution volume, mL; m is the average mass of the three samples before treatment, g; M is the molar mass of *trans*-resveratrol or polydatin, g/mol; φ is the mean moisture content of the three samples, %; and Y is the target analyte yield, μ mol/g.

2.5. Kinetic model

The kinetic curves of IMUSEH, IL-MAE and IL-UAE were fitted using a first-order kinetic model with the following mathematical equations:

$$\ln (Y_e - Y_t) = \ln Y_e - k \cdot t, \quad (2)$$

$$Y_t = Y_e [1 - \exp (-k \cdot t)], \quad (3)$$

where Y_t and Y_e (μ mol/g) are the yield of *trans*-resveratrol at any time t (min) and at equilibrium, respectively, and k (μ mol/g/min) is the mass transfer coefficient of the entire process.

2.6. Process optimization by RSM

The interaction between factors is important to obtain a higher yield, and should be optimized by RSM using Box–Behnken design (BBD) software for data processing. The BBD with three factors was used in Design-Expert 7.0 without any blocking. The boundaries of IMUSEH time, microwave irradiation power, and liquid–solid ratio were 10–30 min, 230–540 W, and 10–30 mL/g, respectively. Specific conditions for each experiment are shown in Table 2.

2.7. SEM

The sample morphology was studied by SEM (Quanta 200; FEI, Hillsboro, OR, USA). Before the examination, unprocessed and processed samples were sputter coated with a thin layer of gold–palladium (5–10 nm, 10 mA, 30 s) at room temperature using a sputter coater.

2.8. FTIR

Unprocessed and processed samples were mixed with 1% KBr powder and were pressed at 5 tons for 2 min to obtain self-supporting disks for FTIR measurements. To obtain an FTIR spectrum, the tablet was scanned by MAGNA-IR560 E.S.P (Thermo, Waltham, MA, USA) at 4000–500 cm^{-1} with a resolution of 4 cm^{-1} .

2.9. Statistical analysis

A one-way analysis of variance was used to determine the significance in yields, and the HPLC analysis results are expressed as means plus/minus a standard deviation.

3. Results and discussion

3.1. Screen of basic ionic liquid

3.1.1. Basic ionic liquid type

The anion and cation of basic ionic liquids influence the physicochemical properties significantly, which affects the IMUSEH process. The effects of changing the anion and alkyl chain length of the cation of basic ionic liquids on polydatin yield, *trans*-resveratrol yield and the percentage of *trans*-resveratrol yield in the total yield of polydatin and *trans*-resveratrol (%) obtained (*trans*-resveratrol occupation compared with the sum) were investigated, under the experimental conditions: 0.5 M for different ionic liquids and sodium hydroxide aqueous solution, 20 mL/g for liquid–solid ratio, 385 W for microwave-irradiation power and 20 min for time.

Because of the significant effects of anions on the properties of ionic liquids as described in the literature (Du et al., 2007), 1-butyl-3-methylimidazolium ionic liquids were compared with various anions (Im^- , OH^- and OAc^-) that were commonly used basic ionic liquids and sufficiently hydrophilic to dissolve in water in any proportion. Recently, research has reported on the application of basic ionic liquid in microcrystalline cellulose hydrolysis and the synthesis of biodiesel (Parveen et al., 2016; Zhang et al., 2016); the anion of an ionic liquid can influence pK_b and thus the catalytic properties (Chen et al., 2008). As shown in Figure 3a, a big difference in yield of *trans*-resveratrol between the selected ionic liquids was observed. The yield of *trans*-resveratrol obtained by Im^- was higher than that achieved by OH^- and

OAc⁻ with the same cation. *trans*-Resveratrol occupation compared with the sum obtained by Im⁻ was much higher than that of the other two anions, which indicates that Im⁻ can yield a high hydrolysis rate. The high yield indicates that the alkalinity of Im⁻ was strongest (Du et al., 2007) and it had better extraction ability. An ionic liquid based on Im⁻ was the first option as solvent in subsequent experiments.

In general, the cation moiety is considered to influence ionic liquid properties. Its influence has been studied by investigating a series of water-soluble *N*-methylimidazolium cations with increasing alkyl chain length from C₂ to C₁₀ and the same Im⁻ anion. The result obtained using various ionic liquids as shown in Figure 3b indicates that the *trans*-resveratrol yield increased significantly with increasing alkyl chain length from ethyl to butyl; however, the yield of *trans*-resveratrol decreased when the alkyl chain length increased from butyl to decyl. A slight enhancement and distinct decrease of *trans*-resveratrol occupation compared with the sum was observed with increasing alkyl chain length from ethyl to butyl and then from butyl to decyl. All the basic ionic liquids tested are water-soluble, the hydrophilicity of ionic liquid is increasingly poor, and the hydrophobicity (lipophilicity) gradually increases with the cation alkyl chain increasing (Du et al., 2009). Thus the phenomenon may be explained by the fact that the solubility of butyl with target analytes is more efficient than that of ethyl. However, the steric clash increases with further increase in alkyl chain length from butyl to decyl, according to the previous studies (Liu et al., 2011; Yang et al., 2011). The other reason for the highest yield of *trans*-resveratrol obtained with butyl as cation may be due to that the hydrolysis rate of polydatin to *trans*-resveratrol was higher than others, which was evidenced by the highest *trans*-resveratrol occupation compared with the sum obtained using butyl as

cation. By taking the extraction yield and hydrolysis rate for *trans*-resveratrol into account, $C_4C_1im^+$ was selected for subsequent parameter optimization studies.

Water, ethanol, and sodium hydroxide (0.5 M) were selected for comparison. As shown in Figure 3a, the higher yield of *trans*-resveratrol and lower yield of polydatin obtained with $[C_4C_1im]Im$ than ethanol reveals the advantages of the hydrolysis ability of $[C_4C_1im]Im$, which can be also evidenced by the high *trans*-resveratrol occupation compared with the sum. The yield of *trans*-resveratrol obtained by $[C_4C_1im]Im$ was higher than that achieved using sodium hydroxide as a catalyst. The *trans*-resveratrol occupation compared with the sum of $[C_4C_1im]Im$ was slightly higher than that of sodium hydroxide, with a similar hydrolysis rate. Thus, the high yield of *trans*-resveratrol achieved by $[C_4C_1im]Im$ is mainly attributed to the ionic liquid properties, including better extraction ability, microwave absorption ability, and target analytes dissolution ability. Compared with deionized water, higher yields of target analytes were obtained by $[C_4C_1im]Im$ ionic liquids because of the low dissolution of *trans*-resveratrol and polydatin in water.

Therefore, $[C_4C_1im]Im$ was selected as the best basic ionic liquid in subsequent experiments.

3.1.2. Ionic liquid concentration

Selecting an appropriate concentration of $[C_4C_1im]Im$ is important to obtain the desired yield. The optimal concentration of $[C_4C_1im]Im$ in aqueous solution to obtain *trans*-resveratrol was sought by studying $[C_4C_1im]Im$ solutions of different concentrations (from 0.00 M to 1.25

M) at 20 min IMUSEH time, 385 W microwave irradiation power, and 20 mL/g liquid–solid ratio. As shown in Figure 3c, with changing [C₄C₁im]Im concentration from 0.00 M to 0.50 M, the yield of *trans*-resveratrol increased significantly, but the change was insignificant when the concentration increased further from 0.50 M to 1.00 M. When the [C₄C₁im]Im concentration reached 1.25 M, an obvious reduction in *trans*-resveratrol yield was obtained. By contrast, the polydatin increased gradually and then decreased when the concentration increased from 0.00 M to 1.00 M, and 1.00 M to 1.25 M, respectively. In addition, the yield of *trans*-resveratrol occupation compared with the sum of [C₄C₁im]Im concentration from 0.25 M to 1.25 M was similar except for the [C₄C₁im]Im concentration of 0.00 M.

The yield of *trans*-resveratrol was the sum of the extraction yield and hydrolysis yield from *trans*-resveratrol glycosides (including polydatin), while the yield of polydatin was equal to the extraction yield minus the hydrolysis yield of polydatin to *trans*-resveratrol. Therefore, when the [C₄C₁im]Im concentration increased, the increasing yield of *trans*-resveratrol was attributed to the rapid enhancing extraction yield and hydrolysis yield, while the increasing yield of polydatin may be due to the higher improvement of extraction yield than hydrolysis yield. It is speculated that an appropriate increase in ionic liquid concentration may contribute to enhancing the capabilities of microwave absorption and microwave conversion and improving the solubility of target analytes, thus is conducive to the extraction and hydrolysis. With further increase in [C₄C₁im]Im concentration, the phenomenon of non-obvious decrease or even rapid reduction of *trans*-resveratrol yield and decreased polydatin yield might be explained by that a further increase in [C₄C₁im]Im concentration with high capability of microwave absorption may lead to the degradation or isomerization of target analytes, which negatively influences the

yield of *trans*-resveratrol and polydatin. In addition, polydatin with larger polarity and better solubility in water was subjected to less effect by the [C₄C₁im]Im with high concentration than *trans*-resveratrol. Therefore, the different turning point concentrations for *trans*-resveratrol and polydatin were speculated by that the *trans*-resveratrol was sensitive to the concentration than polydatin, and the dissolution of *trans*-resveratrol in [C₄C₁im]Im with high concentration was lower than polydatin. Thus, 0.50 M was selected as a suitable concentration for subsequent experiments in terms of cost savings.

3.2. Process optimization using factorial design

Some factors potentially influencing the IMUSEH process, including microwave irradiation power, IMUSEH time and liquid–solid ratio, were optimized systematically to obtain a higher yield of *trans*-resveratrol. Each experiment was performed in triplicate.

3.2.1. Microwave irradiation power

Microwave irradiation power is a critical parameter that influences the yield significantly, and experiments were conducted by treating materials using 0.5 M [C₄C₁im]Im dual solvent–catalyst with a 20 mL/g liquid–solid ratio at different microwave-irradiation powers (120, 230, 385, 540, and 700 W) for 20 min. The result obtained is shown in Figure 4a and indicates that the *trans*-resveratrol yield increased rapidly with increasing microwave irradiation power from 120 W to 385 W, but the tendency was opposite with further increase in power. A slight change in *trans*-resveratrol occupation compared with the sum obtained under various irradiation powers was observed. The reason for this tendency is that

microwave irradiation energy can improve solvent penetration into materials by molecular interaction within the electromagnetic field, and provide a rapid transfer of energy between solvent and plant matrix (Liu et al., 2011). However, a high irradiation power may lead to carbonization of plant materials because of internal overheating, isomerization, or thermal instability of analytes. To obtain a higher yield and saving energy, 230–540 W was selected for further optimization.

3.2.2. IMUSEH time

The influence of IMUSEH time on *trans*-resveratrol yield was investigated by treating materials using a 0.5 M [C₄C₁im]Im dual solvent–catalyst with 20 mL/g liquid–solid ratio for various IMUSEH times (5, 10, 15, 20, 30, 40, 50, and 60 min) at 385 W. Figure 4b shows that the yields of polydatin and *trans*-resveratrol increased gradually with increase in time from 5 to 10 min, but then a continued enhancement of *trans*-resveratrol yield and reduction in polydatin yield resulted with an increase in IMUSEH time from 10 min to 20 min. The yields of polydatin and *trans*-resveratrol were almost constant with further increase in time from 20 min to 60 min. In addition, *trans*-resveratrol occupation compared with the sum increased obviously with an increase in IMUSEH time from 5 min to 20 min, but the value was almost constant with further increases in IMUSEH time. The increase in *trans*-resveratrol yield is attributed mainly to the extraction within 10 min. When the time increased above 10 min, the improvement in *trans*-resveratrol yield resulted not only from the extraction, but also from polydatin hydrolysis. The result implied that sufficient time is required to obtain a high *trans*-resveratrol yield, including penetration of the solvent into the materials, dissolution of

target analytes, diffusion of solvent with target analytes outside of the materials, and hydrolysis of polydatin to *trans*-resveratrol. Therefore, 10–30 min was selected in subsequent experiments.

3.2.3. Liquid–solid ratio

Optimization of the liquid–solid ratio used during IMUSEH is significant. The effect of this variable was examined at different liquid–solid ratios (10, 20, 30, 40, and 50 mL/g) with 0.5 M [C₄C₁im]Im as the dual solvent–catalyst, 385 W microwave irradiation power, and 20 min IMUSEH time. From Figure 4c, the yield of *trans*-resveratrol was improved for a liquid–solid ratio from 10 mL/g to 20 mL/g, and was kept almost constant when the ratio increased further. *trans*-Resveratrol occupation compared with the sum for these liquid–solid ratios was similar. Large solvent volumes could lead to difficult procedures and unnecessary waste, whereas small volumes may result in incomplete extraction. To obtain a higher yield of *trans*-resveratrol, conserve solvent, and avoid bulky handling in subsequent processing, a liquid–solid ratio between 10 and 30 mL/g was selected and optimized in subsequent experiments.

3.3. Parameter optimization by RSM

3.3.1. Statistical analysis and model building

The interaction between important parameters should be investigated by RSM, including liquid–solid ratio, microwave irradiation power, and IMUSEH time. As shown in Table 2, experimental data obtained and the result of each dependent variable with the coefficients of

determination (R^2 of 0.98) indicates that the model was significant because the model F -value was 31.56, and there is < 0.01% chance that a “Model F -value” this large could occur because of statistical noise. A “Lack of fit” F -value of 4.98 and a P -value of 7.75% implies that the “Lack of fit” was insignificant and the probability for the occurrence of this “Lack of fit F -value” was 7.75%, which can be treated as statistical noise. In general, the value of the “Probability > F ” less than 0.05 or greater than 0.10 was designed as a significant or insignificant model term, respectively. In this case, X_1 , X_2 , X_1^2 , X_2^2 , and X_3^2 were significant model terms and X_3 , X_1X_2 , X_1X_3 , and X_2X_3 were not. The “Adequacy precision” of 15.14 indicates that this model could be used to navigate the design space because a signal-to-noise ratio greater than 4 was desirable. The following equation was proposed to calculate the final yield of *trans*-resveratrol:

$$Y = -2.913 + 0.164 X_1 + 0.122 X_2 + 0.013 X_3 - 0.003 X_1^2 - 0.003 X_2^2. \quad (4)$$

3.3.2. Diagnostics plots of model adequacy

A determination of the model adequacy is necessary, and various diagnostic plots, including predicted versus actual, normal % probability, and internally studentized residuals were used. From Figure 5a, all points close to a straight line in the plot of predicted versus actual imply that the predicted values obtained by the model derived conformed to the experimental values. In Figure 5b, the normal % probability plot of residuals for response tended to be a straight line, which indicates that it was normally distributed and showed no deviation in variance. Figure 5c (plot of internally studentized residuals) shows that all data points lie within the limits (± 3), and implies that the developed model possesses a

satisfactory fit. Therefore, the model was reliable to fit interactions between the different factors.

3.3.3. Process variables influencing *trans*-resveratrol yield

3.3.3.1. Perturbation plot

Three parameters were selected to optimize the RSM with differences in the influencing degrees of various parameters on *trans*-resveratrol yield. A perturbation plot that was plotted by the response (yield of *trans*-resveratrol) according to the deviation from the reference point of one parameter but with all other factors constant was used to evaluate the influence of various factors. The importance of factors was reflected in the curvilinear shape. A steeper or more curved curvilinear shape indicates a factor of greater importance. In contrast, a factor with a relatively flat line implies that the factor was relatively unimportant (Moura et al., 2010). The curves of the three parameters in Figure 6 show that the microwave irradiation power with a curve with a large camber has an important influence on *trans*-resveratrol yield. However, the effect of the liquid–solid ratio and IMUSEH time on *trans*-resveratrol yield was similar and smaller compared with that of the microwave irradiation power.

3.3.3.2. Interpretation of response surface models

Response surfaces for the independent or interactions effect of variables on *trans*-resveratrol yield are shown in Figure 7. The interaction of liquid–solid ratio (X_1) and IMUSEH time (X_2) with a fixed microwave irradiation power (0 level) is presented in Figure 7a. The *trans*-resveratrol yield increased gradually and then remained constant with increase in

liquid–solid ratio. The variation in trend of *trans*-resveratrol yield with increase in IMUSEH time was similar to that of the liquid–solid ratio; it increased first and was followed by an inconspicuous enhancement in yield. The three-dimensional response surface plotted at various liquid–solid ratios (X_1) and for microwave irradiation powers (X_3) with a fixed IMUSEH time (0 level) is shown in Figure 7b. As the microwave irradiation power increased, the *trans*-resveratrol yield increased and then decreased, which indicates that microwave irradiation powers above or below the optimal level would decrease the yield. The initial increase in *trans*-resveratrol yield was followed by an inconspicuous improvement with increasing liquid–solid ratio. Figure 7c shows the interaction of IMUSEH time (X_2) and microwave irradiation power (X_3) with a fixed liquid–solid ratio (0 level). The yield of *trans*-resveratrol increased significantly and then remained constant with increase in IMUSEH time. When the microwave irradiation power increased, the *trans*-resveratrol yield increased first, but the initial increase in *trans*-resveratrol yield was followed by an obvious decrease.

Software predictions of optimal conditions were a liquid–solid ratio of 24 mL/g, IMUSEH time of 25 min, and microwave irradiation power of 396 W. Under optimal conditions, the theoretical yield of *trans*-resveratrol was 3.11 $\mu\text{mol/g}$.

3.4. Verification tests

Verification tests were performed by treating samples three times under optimal conditions derived in the RSM study (0.5 M [C₄C₁im]Im, 24 mL/g liquid–solid ratio, 25 min IMUSEH time, and 396 W microwave irradiation power). The actual yield of *trans*-resveratrol was 3.06

μmol/g with an error of 0.12 μmol/g, which confirmed the theoretical *trans*-resveratrol yield.

So far, the *trans*-resveratrol was usually obtained from a variety of berries, *Rhizma Polygoni Cuspidati* and peanuts (Aggarwal et al., 2004). For *Rhizma Polygoni Cuspidati*, the most important resource for obtaining *trans*-resveratrol, the content of *trans*-resveratrol was found to be 2.42-2.65 mg/g based on the reference (Du et al., 2007). The content of *trans*-resveratrol in peanuts ranged from 0.03 to 0.14 μg/g (Sanders et al., 2000). In addition, reference reported that *trans*-resveratrol existed in red and white grape skins with the content ranged from 11.1 to 123.0 μg/g (Kammerer et al., 2004), and the amounts of *trans*-resveratrol in various kinds of grapes were existed with 0.09-2.92 μg/g (Piñeiro et al., 2006). Although the content of *trans*-resveratrol (0.70 mg/g) obtained in this study was much lower than that in *Rhizma Polygoni Cuspidati*, which was found to be higher than other resources. Therefore, application of the tree peony seed oil-extracted residues for obtaining *trans*-resveratrol is very meaningful for the full utilization of resources.

3.5. Method validation

3.5.1. Linearity, limit of detection, and quantification

A linear calibration curve equation was drawn by peak area (Y) versus concentration (X), and linear equations of *trans*-resveratrol and polydatin were $Y_{trans-resveratrol} = (1551968 \pm 29007) + (68374016 \pm 642716) X$ ($r^2 = 0.9998$, $n = 7$), and $Y_{polydatin} = (766938 \pm 15241) + (31434756 \pm 270339) X$ ($r^2 = 0.9992$, $n = 7$), respectively. The calibration curves showed good linearity for *trans*-resveratrol and polydatin from 0.01 to 0.52 mg/mL and 0.01 to 0.55 mg/mL, respectively.

Limit of detection (LOD) for the determination of polydatin and *trans*-resveratrol under the present chromatographic conditions was calculated based on the standard deviation of Y-intercept of the regression line (α) and the slope (S), using the following equation $LOD = 3.3 \alpha/S$. Limit of quantification (LOQ) was calculated by the equation $LOQ = 10 \alpha/S$. The LOD and LOQ to determine *trans*-resveratrol were 1.4 and 4.2 $\mu\text{g/mL}$, and to polydatin were 1.6 and 4.8 $\mu\text{g/mL}$, respectively.

3.5.2. Stability

To investigate the stability of *trans*-resveratrol in $[\text{C}_4\text{C}_1\text{im}]\text{Im}$ aqueous solution, experiments were performed by treating the standard under optimal conditions and 1 week later. The *trans*-resveratrol stability was assessed by the recovery of *trans*-resveratrol under the obtained optimal conditions. Table 3 shows recoveries of 99.22% and 98.83% after 7 days with no change in retention time. Therefore, no obvious degradation was observed and *trans*-resveratrol was stable in $[\text{C}_4\text{C}_1\text{im}]\text{Im}$ aqueous solution under the selected optimal conditions.

3.5.3. Recovery

Three concentrations of *trans*-resveratrol standard solution were added into tree peony seed oil-extracted residues, which were treated by IMUSEH under the optimal conditions to evaluate the accuracy of the proposed method. From Table 4, the mean recovery of *trans*-resveratrol from tree peony seed oil-extracted residues was 97.68%.

3.5.4. Repeatability

Experiments to treat powders under the optimal conditions were performed five times to evaluate the repeatability of the developed method. The mean yield of *trans*-resveratrol obtained with a calculated relative standard deviation of 2.8% indicates that the developed technique has a good repeatability.

The proposed IMUSEH method is credible to prepare *trans*-resveratrol because good repeatability, precision, and stable *trans*-resveratrol existed in 0.5 M [C₄C₁im]Im aqueous solution.

3.6. IMUSEH mechanism

In order to highlight the mechanism of the microwave–ultrasonic synergistic apparatus, the kinetic curves of IMUSEH, IL-MAE and IL-UAE were plotted as the yield of *trans*-resveratrol versus time. Experiments were carried out based on optimal conditions excluding time. Figure 8 shows an R^2 of 0.9746, 0.9609 and 0.9835 for the three methods, which indicates that the experimental data agree reasonably with the predicted results achieved by the first-order kinetic model. For the developed IMUSEH, in the first 20 min, a significant improvement in *trans*-resveratrol yield was observed, but the yield was enhanced slightly with a further increase in time. When the time reached 20 min, the *trans*-resveratrol yield reached 90.45% of the equilibrium yield obtained by the first-order kinetic models (3.26 $\mu\text{mol/g}$). For IL-MAE, the yield of *trans*-resveratrol decreased obviously, and the time required to reach equilibrium was extended to 30 min. In addition, the yield obtained by IL-UAE was much lower than that by IMUSEH, and almost 40 min was required to reach the equilibrium of *trans*-resveratrol yield for IL-UAE.

The difference between the three techniques is attributed to the mechanism of the microwave–ultrasonic synergistic apparatus. A higher yield and efficiency were achieved by IMUSEH because of the synergetic effect between the molecular force induced by microwave and the mechanical force induced by ultrasound. The high frequency electromagnetic wave of microwave irradiation can penetrate medium to reach the inside of plant materials, and then can be converted into heat energy to heat materials instantaneously and homogenously. Cell walls will be ruptured when internal pressure of cell is beyond the bearing ability of cell wall, which contributing to the leaching of the components into the surrounding solvent (Mandal et al., 2007; Wang and Weller, 2006). Therefore, microwave irradiation can increase the extraction efficiency since the enhancing mass diffusivity of bioactive compounds from within the plant matrix to the outside solvent. Cavitation phenomenon is the main extraction mechanism of ultrasound-assisted extraction process. Cavitation caused by ultrasound waves between solvent and samples can result in bubble collapse, cause extra sample vibration, and improve contact between the sample matrix and the liquid solvent phase (Pan et al., 2010; Zu et al., 2012). In addition, the pressure produced by the cavitation can disrupt cell walls and increase the permeability, thus enhancing the mass transfer of cell contents from the solid to the liquid phase.

Therefore, the combination of microwave and ultrasound in microwave–ultrasonic synergistic apparatus could make the microwave molecular force and the ultrasound mechanical force occur simultaneously. The collapse of cell walls, leaching of bioactive compounds and sample vibration were performed continuously, leading to a higher yield and efficiency.

3.7. Structural changes after IMUSEH

To assess the developed IMUSEH technique further, physical structural alterations in residue samples before and after IMUSEH were analyzed and investigated by SEM, compared with IL-MAE and IL-UAE. Figure 9 shows a large number of intact cells of raw material and internal cells were disorganized and broken more completely by the IMUSEH method. By contrast, a partial destruction of cell walls occurred with part of them remaining integrated after IL-MAE and IL-UAE. The damage degree of cell walls by IL-UAE was less than that by IL-MAE, which was attributed to the different mechanisms of microwave and ultrasound-assisted extraction process. Severe thermal stresses and localized high pressures in microwave heating could contribute to the rapid rupture of cell walls and heat transfer is provided by radiation, conduction, and convection in microwave irradiation (Wu et al., 2009). Constant disturbance by ultrasound irradiation can facilitate even sample heating, which leads to the exposure of target analytes in dual solvent–catalyst $[\text{C}_4\text{C}_{1\text{im}}]\text{Im}$ and a higher yield of *trans*-resveratrol.

Chemical structure changes in raw residue materials and materials treated by IMUSEH, IL-MAE and IL-UAE were analyzed by FTIR from 400 to 4000 cm^{-1} as shown in Figure 10. Reflectance peaks between 1800 and 700 cm^{-1} were selected as representative peaks for known carbohydrate compounds (Liu et al., 2011). No obvious change in peak positions and intensities at 1746, 1652, 1541, and 1053 cm^{-1} were observed before and after treatment based on FTIR spectra. This indicates that the chemical structures of sample matrixes, including polysaccharides and protein, were not broken obviously after treatment by the three

methods. The alkaline condition produced by the IMUSEH method is insufficient to result in a significant degradation of polysaccharide, but it can break the glucosidic bond of *trans*-resveratrol glycosides selectively to free *trans*-resveratrol. The result implies that the IMUSEH condition is relatively moderate.

4. Conclusion

A novel and efficient procedure to obtain *trans*-resveratrol from tree peony seed oil-extracted residues by imidazolium-based ionic liquid based microwave-ultrasonic synergistic simultaneous extraction and hydrolysis (IMUSEH) method was presented. The highest yield of *trans*-resveratrol ($3.06 \pm 0.12 \mu\text{mol/g}$) was obtained at 0.5 M [C₄C₁im]Im, 24 mL/g liquid–solid ratio, 25 min IMUSEH time, and 396 W microwave irradiation power. [C₄C₁im]Im with a short C₄ alkyl side-chain on the imidazoline ring and imidazolidine as anion has excellent solvent–catalytic performance. Kinetics curves showed that IMUSEH is a more efficient method to obtain *trans*-resveratrol. *trans*-Resveratrol was stable in the [C₄C₁im]Im dual solvent–catalyst under optimal conditions and the proposed method also showed a high reproducibility. Thus, the proposed effective IMUSEH method shows great promise to transform glucoside to aglycone in natural products.

Acknowledgements

The authors gratefully acknowledge the financial supports by the Special Fund for Forestry Scientific Research in the Public Interest (20140470102) and the National Science Foundation of China (Grant no. NSFC31670580).

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Figure captions

Figure 1. Schematic diagram of the experimental apparatus.

Figure 2. Comparative HPLC chromatograms for polydatin and *trans*-resveratrol in standard solutions (a), an extract obtained using 0.5 M [C₄C₁im]Im as dual solvent–catalyst (b) and using ethanol as extraction solvent (c). Inset: schematic of the transformation process of the polydatin to free *trans*-resveratrol.

Figure 3. Effect of common solvents and basic ionic liquids with different anions (a) and cation carbon chain lengths (b), and basic ionic liquid concentration (c) on *trans*-resveratrol yield, polydatin yield and *trans*-resveratrol occupation compared with the sum.

Figure 4. Optimization of IMUSEH process using a factorial design. Effect of microwave irradiation power (a), IMUSEH time (b), and liquid–solid ratio (c) on *trans*-resveratrol yield, polydatin yield and *trans*-resveratrol occupation compared with the sum.

Figure 5. Diagnostic plots for model adequacy. (a) Predicted versus actual, (b) normal % probability, and (c) internally studentized residuals.

Figure 6. Perturbation plot showing effect of operational variables.

Figure 7. Optimization of yield of *trans*-resveratrol using BBD. (a) Interaction of liquid–solid ratio and IMUSEH time, (b) interaction of liquid–solid ratio and microwave irradiation power, and (c) interaction of IMUSEH time and microwave irradiation power.

Figure 8. Dynamic curves for *trans*-resveratrol yield obtained by developed IMUSEH,

IL-MAE and IL-UAE. Points represent actual values, and lines represent fitting behavior predicted by first-order models.

Figure 9. Scanning electron microscopy images of tree peony seed oil-extracted residues: (a) untreated sample, (b) IMUSEH, (c) IL-MAE, and (d) IL-UAE.

Figure 10. Fourier-transform infrared spectroscopy of tree peony seed oil-extracted residues: (a) untreated sample, (b) IMUSEH, (c) IL-MAE, and (d) IL-UAE.

Table 1 The structures and properties of basic ionic liquids.

Ionic liquid	Cation	Anion	Appearance	Chemical formula	Solubility in H ₂ O (g/100mL)
[C ₄ C ₁ im]OAc		OAc ⁻	Pale-yellow liquid	C ₁₀ H ₁₈ N ₂ O ₂	Totally miscible
[C ₄ C ₁ im]OH		OH ⁻	Pale-yellow liquid	C ₈ H ₁₆ N ₂ O	Totally miscible
[C ₂ C ₁ im]Im			Orange-red liquid	C ₉ H ₁₄ N ₄	Totally miscible
[C ₄ C ₁ im]Im			Orange-red liquid	C ₁₁ H ₁₈ N ₄	Totally miscible
[C ₆ C ₁ im]Im			Orange-red liquid	C ₁₃ H ₂₂ N ₄	Totally miscible
[C ₈ C ₁ im]Im			Orange-red liquid	C ₁₅ H ₂₆ N ₄	Miscible
[C ₁₀ C ₁ im]Im			Orange-red liquid	C ₁₇ H ₃₀ N ₄	Miscible

Table 2 BBD with experimental and predicted values for *trans*-resveratrol yield, analysis of variance (ANOVA) for response surface quadratic model, and fit statistics for the response values ^a.

Run	BBD experiments				ANOVA								
	X_1^b	X_2	X_3	Y	Source	Sum of squares		Degree of freedom		Mean square	F-value	P-value	
1	10	10	385	1.68	Model ^b	2.96		9		0.33	31.56	< 0.0001 ^c	
2	30	10	385	2.48	X_1	0.70		1		0.70	67.53	< 0.0001 ^c	
3	10	30	385	2.34	X_2	0.63		1		0.63	60.16	0.0001 ^c	
4	30	30	385	3.04	X_3	0.00		1		0.00	0.45	0.5247	
5	10	20	230	2.01	X_1X_2	0.00		1		0.00	0.24	0.6393	
6	30	20	230	2.46	X_1X_3	0.00		1		0.00	0.02	0.8998	
7	10	20	540	2.03	X_2X_3	0.02		1		0.02	1.88	0.2127	
8	30	20	540	2.45	X_1^2	0.43		1		0.43	40.81	0.0004 ^c	
9	20	10	230	2.06	X_2^2	0.30		1		0.30	28.62	0.0011 ^c	
10	20	30	230	2.43	X_3^2	0.72		1		0.72	68.85	< 0.0001 ^c	
11	20	10	540	2.01	Residual	0.07		7		0.01			
12	20	30	540	2.66	Lack of fit	0.06		3		0.02	4.98	0.0775	
13	20	20	385	3.05	Pure error	0.02		4		0.00			
14	20	20	385	2.91	Corrected total	3.03		16					
15	20	20	385	2.92	Credibility analysis of the regression equations								
16	20	20	385	3.02	Index mark	Standard deviation	Mean	Coefficient of variation %	Press	R^2	Adjust R^2	Predicted R^2	Adequacy precision
17	20	20	385	2.95	Y	0.10	2.50	4.08	0.95	0.98	0.95	0.69	15.14

^a Results were obtained using Design Expert 7.0 software.

^b X_1 : liquid–solid ratio (mL/g), X_2 : IMUSEH time (min), X_3 : microwave irradiation power (W), Y: *trans*-resveratrol yield ($\mu\text{mol/g}$).

^c Significant at $P < 0.05$

Table 3 Stability studies of *trans*-resveratrol standard at optimum conditions ($n = 3$) ^a.

Compound	Initial concentration (mg/mL)	Recovered concentration after IMUSEH ^b (mg/mL)	RSD% ($n=3$)	Average recovery (%)	Recovered concentration after 7 day (mg/mL)	RSD% ($n=3$)	Average recovery (%)
<i>trans</i> -Resveratrol	0.516	0.512	0.97	99.22	0.506	0.96	98.83

^a Conditions: 396 W microwave irradiation power, 25 min IMUSEH time, 24 liquid–solid ratio (mL/g), and 0.5 M [C₄C₁im]Im as the dual solvent–catalyst.

^b Imidazolium-based ionic liquid-based microwave–ultrasonic synergistic simultaneous extraction and hydrolysis.

Table 4 Recovery of *trans*-resveratrol from tree peony seed oil-extracted residues ($n = 3$).

Sample	Content of the sample (mg)	Mass of added standard (mg)	Mass of the sample analyzed with added standard (mg)	Recovery (%)
1	3.85	1.00	4.78	98.56
2	3.85	2.00	5.68	97.09
3	3.85	5.00	8.62	97.40
Average				97.68