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Conversion of steroid saponins into diosgenin by catalytic hydrolysis using acid-functionalized ionic liquid under microwave irradiation

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ABSTRACT

The catalytic hydrolysis with an acid-functionalized ionic liquid under microwave irradiation was successfully developed to convert steroid saponins into diosgenin. The typical acid-functionalized ionic liquid, 1-sulfobutyl-3-methylimidazolium hydrosulfate ($[BHSO_3MIm]HSO_4$), was used to evaluate the catalytic efficiency. The results strongly suggested that acid-functionalized ionic liquid under microwave irradiation significantly improved the hydrolysis efficiency of steroid saponins. In addition, we optimized hydrolysis parameters, including the ionic liquid concentration, the ratio of solvent to solid, the reaction temperature, and reaction time. Under the optimal conditions, this approach achieved the highest yield of diosgenin (29.97 ± 0.51 mg) with 0.3 g steroid saponins. Compared with regular hydrochloric acid hydrolysis, the developed approach obtained 96% diosgenin and reduced 93% reaction time, indicating that the catalytic hydrolysis with an acid-functionalized ionic liquid under microwave irradiation had a broad application prospect in diosgenin production.

1. Introduction

The medicinal plant *Dioscorea zingiberensis* C. H. Wright (DZW), which belongs to dioscoraceae family, is widely distributed in Shanxi, Hubei, and Yunnan Provinces in China (Zhang et al., 2012). Its rhizome is a well-known Traditional Chinese Medicine. And it is the main source of diosgenin (Wang et al., 2008), a starting material for the semi-synthesis of drugs of steroidal hormones, such as oral contraceptives, sex hormones, and other steroids (Oncina et al., 2000). In recent years, various medicinal usages of diosgenin, including anti-thrombosis effect (Gong et al., 2011), anti-oxidation (Rajalingam et al., 2012), anti-proliferation, and anti-invasion effects on cancer cells (Mao et al., 2012), have been reported.

In the plants of *Dioscorea Linn.*, diosgenin is stored in the form of saponins, which are regarded as the major active components of DZW (Liu et al., 2010a, b, c). Saponins are connected with glucoses or rhamnoses to aglycone with C–O glucosidic bonds (Zhang et al., 2006). Industrial diosgenin is mainly prepared through hydrochloric acid hydrolysis of these glucosidic bonds. However, a large quantity of wastewater with low pH and the high level of chemical oxygen demand (COD) is discharged during the industrial production of diosgenin, thus leading to serious environmental pollution. Moreover, secondary reactions, such as chlorinated reaction, in inorganic acid hydrolysis decrease the production of diosgenin.

In order to reduce environmental pollution, many hydrolysis technologies for the conversion of steroid saponins to diosgenin have been studied to replace inorganic acid hydrolysis technology. *Trichoderma reesei* (Zhu et al., 2010), *Trichoderma harzianum* (Liu et al., 2010a, b, c), and *Aspergillus oryzae* (Dong et al., 2010) could produce diosgenin with less pollution. Microorganisms synthesize a series of glycosidases to hydrolyze steroid saponins. The direct microbial transformation method of plant materials has been widely employed due to its low cost and comprehensive utilization way of starch in the plant. Starch is not hydrolyzed under acid conditions, but it can be fully utilized by microorganisms under acid conditions. It greatly reduces the emissions of high levels of COD. However, multi-substrates and enzymes in raw herbs during biotransformation were not thoroughly investigated and a high conversion rate was not obtained (Dong et al., 2010). Furthermore,

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biotransformation is non-directional conversion and many intermediate products are produced by microorganisms during biotransformation. A large quantity of organic solvent is consumed during separation and purification of target components. Compared with the traditional method, the biotransformation method usually has the long production cycle of 3–7 days and the low production efficiency. Therefore, many researchers paid their attentions to enzymatic hydrolysis. Enzymatic hydrolysis from saponins to diosgenin was carried out by β-glucosidase from *Aspergillus fumigates* (Lei et al., 2012) or commercial cellulose (Liu et al., 2010a, b, c). Enzymatic hydrolysis of natural products is an effective method and has the following advantages: high specificity, mild reaction conditions, and environment-friendly process. However, this method requires the preparation process of enzymes. However, the low catalytic activity for specific glycosidic bond leads to the low yield. Moreover, this method cannot be applied in industrial production because of its high cost.

Therefore, it is necessary to develop an efficient, convenient, and economical conversion method. In recent years, ionic liquids, as a kind of environment-friendly solvent, have shown great potential in replacing conventional organic solvents in many fields (Wu et al., 2013; Naushad et al., 2012; Yu et al., 2011; Yi et al., 2013). Ionic liquids are composed of various cations and anions and ionic liquids at room temperature are still in the liquid state. Therefore, they are non-volatile and non-flammable and can be miscible with water and various organic solvents (Wilkes, 2004; Boschetti et al., 2007). Ionic liquids show the outstanding design capability compared with conventional solvent systems. Moreover, ionic liquids are recyclable and environmentally compatible and can alleviate environmental pollution. Therefore, we adopted ionic liquid as a "green catalyst" in the hydrolysis reaction. Acid-functionalized ionic liquid shows the excellent catalytic hydrolysis performance and has the characteristics of conventional ionic liquids. In addition, due to the introduction of acid functional groups, it has some other peculiarities, such as uniform acid intensity distribution, high acid density, adjustable acidity, and durable acidity.

In order to shorten reaction time, we used microwave heating method instead of conventional thermal methods. Microwave has attracted considerable attentions because its heating effect can greatly shorten reaction time and significantly improve the rate and yield of reactions (Huang et al., 2006). Ionic liquids are composed of a pair of ions and therefore have a high density of strong dipoles, which make them promising candidates for microwave absorption (Shih et al., 2011).

To the best of our knowledge, acid-functionalized ionic liquid catalytic hydrolysis of steroid saponins under microwave irradiation was not reported. In this study, we combined ionic liquids with microwave technologies to achieve the efficient and clean production of diosgenin (Fig. 1). In addition, important reaction factors, such as ionic liquid concentration, the ratio of solvent to solid, and hydrolysis time, were studied. The recovery method was also investigated. All solvents used in hydrolysis are environment-friendly and recyclable. Finally, this method was compared with the traditional method to highlight its advantages.

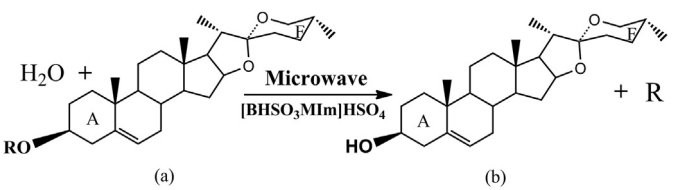

Fig. 1. Diosgenin production by acid-functionalized ionic liquid $[BHSO_3MIm]HSO_4$ under microwave irradiation. (a): Steroidal saponins. (b): Diosgenin. R: A number of sugars, such as glucoses and rhamnoses.

2. Experimental

2.1. Materials

D. zingiberensis C. H. Wright rhizomes were dried at 50 °C and then crushed into powder. The preparation method of total saponins was based on the report by Yang et al. (2003). Dry rhizome powder was mixed with 70% aqueous EtOH and heat reflux extraction was carried out twice at 60 °C. Then the extraction solution was filtered, and concentrated in the rotary evaporator to recover ethanol. The rest aqueous solution was partitioned with *n*-BuOH for three times to obtain saponin extracts. Then *n*-BuOH was recovered and total steroid saponins were obtained.

Acid-functionalized ionic liquid 1-sulfobutyl-3-methylimidazolium hydrosulfate ($[BHSO_3MIm]HSO_4$, purity > 99%) was purchased from Lanzhou Institute of Chemical Physics (Gansu). Diosgenin standard was purchased from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai). The purity of standard compound was higher than 99%.

Chromatographic grade of acetonitrile was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). All the solvents prepared for HPLC (high-performance liquid chromatography) were filtered through the 0.22-μm microporous membrane.

Petroleum ether and *n*-BuOH were all of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai).

2.2. Apparatus

XH-800B intelligent microwave workstation with an output power of 0–1000 W was purchased from Beijing Xianghu Science and Technology Development Co., Ltd. (Beijing). The temperature of the reaction mixture was monitored and kept constant (±1 °C). A positive & negative controlled rotary plate was placed on the floor of the microwave cavity and six samples could be processed at the same time. In the actual experiments, the reaction temperature was kept constant through fluctuant microwave power.

The Agilent 1100 series HPLC system was purchased from Agilent (California, USA). A GraceSmart RP C18 column (5 μm, 4.6 × 250 mm. W. R. Grace & Co.-Conn, Columbia, Maryland, USA) was used.

2.3. Optimization of microwave-assisted hydrolysis

In order to get the optimal hydrolysis, four experimental parameters, the concentration of ionic liquid, the ratio of solvent to solid, the reaction temperature, and reaction time, were investigated and optimized by univariate analysis method. In the experiments, ionic liquid $[BHSO_3MIm]HSO_4$ was fully dissolved in deionized water in a beaker, transferred to a volumetric flask, and diluted to different concentrations. Then 0.3 g steroid saponins were weighed, added into a reaction tank, and then mixed with a certain volume of ionic liquid. Reactions were carried out in a microwave cavity by the pre-set program with different parameters. The instrument automatically started the cooling process immediately after the reaction was completed. After cooling, the solution was filtered and washed with distilled water for several times. The filtrated residue was dried at 50 °C, and then extracted in a soxhlet extractor for 3 h with petroleum ether. The solvent extracts were concentrated and dried in a rotary evaporator. Then diosgenin was dissolved with methanol and then filtered through the 0.22-μm membrane. Diosgenin was determined by HPLC.

2.4. Recycling experiment

Ionic liquids are recyclable. Therefore, ionic liquid recovery method was also studied. After each reaction, reaction products,

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insoluble diosgenin, and saponins in water were removed by filtration. Then filtrate was partitioned twice with saturated *n*-BuOH. The *n*-BuOH layer was concentrated and dried to recover the solvent in a rotary evaporator and obtain the unreacted or intermediate saponins. The *n*-BuOH and saponins were recycled. The bottom layer is a mixture of water, ionic liquid and less impurities. After removing water and vacuum drying for half an hour, the slightly yellow ionic liquid was obtained and used in the next reaction. Moreover, the recyclability of $[BHSO_3MIm]HSO_4$ was investigated through the reactions for five times.

2.5. Conventional method

In order to highlight the effects of the $[BHSO_3MIm]HSO_4$ and microwave irradiation, conventional hydrochloric acid hydrolysis in oil bath or microwave was selected as the control for comparison. According to conventional oil bath hydrolysis method, 0.3 g saponins was mixed with 10 mL of 2 mol L^{-1} hydrochloric acid and then hydrolyzed at 100 °C for 5 h in oil bath (Liu et al., 1995). According to conventional microwave hydrolysis method, 0.3 g steroid saponins were mixed with 2 mol L^{-1} hydrochloric acid (4.5 mL) and then hydrolyzed at 100 °C for 20 min under microwave irradiation. All the samples were analyzed under the same conditions with microwave-assisted hydrolysis.

2.6. HPLC analysis

The HPLC quantitative analysis for diosgenin was performed on the Agilent 1100 series. The sample (10 μL) was injected into C18 column at 30 °C, and the flow rate was kept at 1.0 mL min^{-1}. The optimized mobile phase composition was acetonitrile-water (95:5, v/v). The regression equation and correlation coefficient (R^2) of diosgenin were determined and derived as $y = 4418.6x-41.792$ ($R^2 = 0.9999$. x: the concentration of diosgenin. y: the corresponding peak area).

3. Results and discussion

The acid-functionalized ionic liquid catalytic hydrolysis from steroid saponins to diosgenin under microwave irradiation was evaluated by changing the ionic liquid concentration, the ratio of solvent to solid, the reaction temperature, and reaction time.

3.1. Effect of the ionic liquid concentration

The concentration of the acid-functionalized ionic liquid is the dominant factor of the hydrolysis. To obtain the maximum yield of diosgenin and to observe the effect of ionic liquid concentration on the hydrolysis reaction, ionic liquid concentration range was set as 1–6 mol L^{-1}. As shown in Fig. 2, the yield of diosgenin increased significantly when the concentration increased from 1 to 3 mol L^{-1} and increased slowly when the concentration increased from 3 to 4 mol L^{-1}. However, the yield of diosgenin decreased when the concentration went up to 5 mol L^{-1}. Moreover, compared to lower concentrations, the higher concentration accelerated the decrease of diosgenin. These significant changes indicated that ionic liquid had an optimal concentration range for the hydrolysis under microwave irradiation. The lower concentration may lead to the slow hydrolysis rate and low yield of diosgenin. And the lower concentration of ionic liquid can only convert steroid saponins into some intermediate products, such as diosgenin-triglucoside, diosgenin-diglucoside, and trillin (Tang and Eisenbrand, 1992). However, the high concentration can increase the side reaction and affect the yield of diosgenin. Diosgenin is easily dehydrated to Δ3, 5-deoxytigogenin (Chen and Wu, 1994; Tang et al., 2013) in the high concentration of ionic liquid. In addition, the high concentration of acid-functionalized ionic liquid resulted in diosgenin C25 epimerization and some changes in F ring (Yang and Chen, 1983). Therefore, the appropriate concentration of $[BHSO_3MIm]HSO_4$ should be determined in advance. And 4 mol L^{-1} $[BHSO_3MIm]HSO_4$ was used in subsequent reactions according to the experimental results.

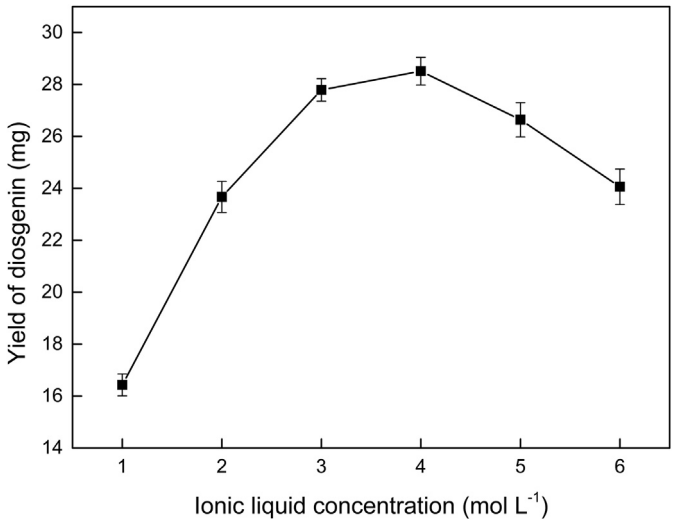

Fig. 2. Effect of the ionic liquid concentration on yield of diosgenin. Reactions were carried out in different concentrations of $[BHSO_3MIm]HSO_4$ (6 mL). 0.3 g steroid saponins were mixed with ionic liquid and hydrolyzed at 100 °C for 15 min under microwave.

3.2. Effect of the reaction temperature

The reaction temperature is another parameter that directly affects reaction products (Guo et al., 2013). The influence of reaction temperature on the yield of diosgenin was explored under five different temperatures (80 °C, 90 °C, 100 °C, 110 °C, and 120 °C). As shown in Fig. 3, the optimum reaction temperature is 100 °C. When the temperature was increased from 80 to 100 °C, the yield of diosgenin was increased significantly. Temperature rise accelerated the reaction (Hu et al., 2013) and enhanced the $[BHSO_3MIm]HSO_4$ catalytic effect. In this temperature range, side reaction was not significant and diosgenin could safely exist in the reaction system. However, side reactions were also enhanced when the reaction temperature increased. Diosgenin became unstable in the high-temperature solution and its structure could be destroyed. Dehydration, cyclization, double-bond shift, and conformational change at 100 °C were studied in the following experiments.

It is noted that the temperature rise process under microwave heating is different from that of the conventional heating method, such as oil bath heating. The temperature increased rapidly in the first heating stage with high microwave power. When the temperature gradually approached the pre-set temperature, the microwave power was gradually reduced. Finally, the temperature was remained at a predetermined temperature by constantly changing microwave power. Microwave thermal effect made the heating process quick and simple. As a polar solvent, $[BHSO_3MIm]HSO_4$ ionic liquid can fully absorb microwave and be heated in the microwave field (Hoffmann et al., 2003).

3.3. Effect of the ratio of solvent to solid

The solvent volume has an important influence on the yield of diosgenin during the hydrolysis process. The appropriate volume

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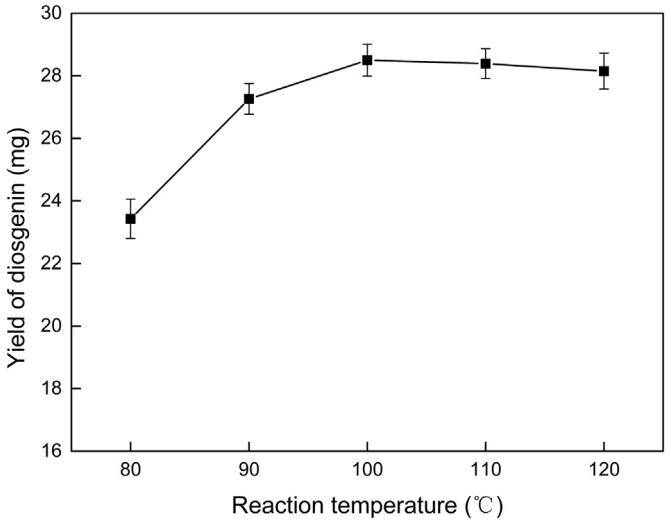

Fig. 3. Effect of the reaction temperature on yield of diosgenin. Reactions were carried out in 4 mol L^{-1} $[BHSO_3MIm]HSO_4$ (6 mL). 0.3 g steroid saponins were mixed with ionic liquid and hydrolyzed at different temperatures for 15 min under microwave.

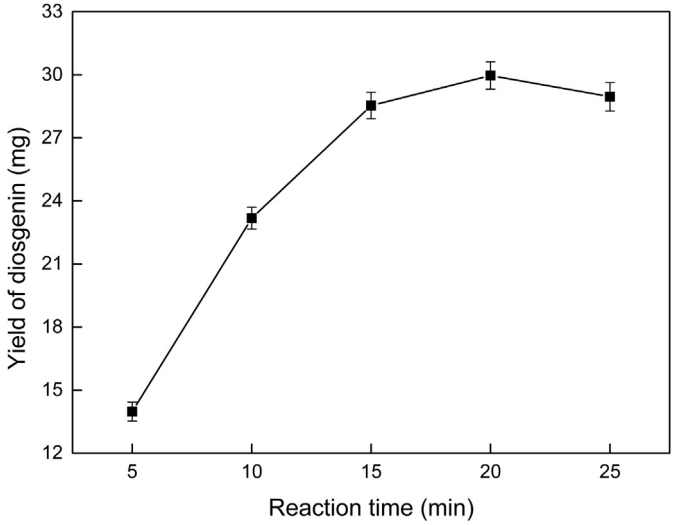

Fig. 5. Effect of the reaction time on yield of diosgenin. Reactions were carried out in 4 mol L^{-1} $[BHSO_3MIm]HSO_4$ (4.5 mL). 0.3 g steroid saponins were mixed with ionic liquid and hydrolyzed at 100 °C for different time.

of solvent can completely submerge the materials without causing waste (Zhu et al., 2013; Lu et al., 2011). In this paper, five different ratios of solvent to solid (5:1, 10:1, 15:1, 20:1 and 25:1) with 4 mol L^{-1} $[BHSO_3MIm]HSO_4$ were investigated to evaluate the influence of solvent volume. As shown in Fig. 4, the yield of diosgenin increased when the ratio of solvent to solid was changed from 5:1 to 10:1. Then it increased slowly when the ratio of solvent to solid was changed from 10:1 to 15:1. The difference in extraction yield was not significant in the range from 15:1 to 20:1. However, it decreased slightly when the ratio went up to 25:1. The decrease may be interpreted as follows: the excessive acid solution changes the diosgenin structure. In addition, excessive solvent increases the difficulty of subsequent steps. A ratio of solvent to solid of 15:1 was selected in subsequent experiments.

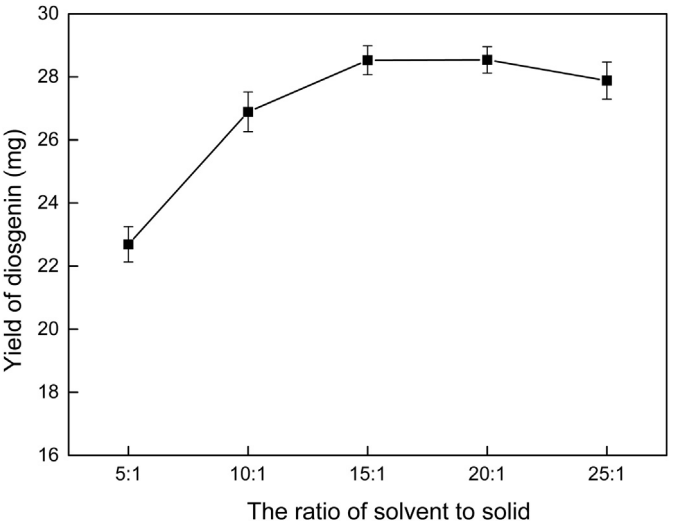

Fig. 4. Effect of the solvent to solid ratio on yield of diosgenin. Reactions were carried out in 4 mol L^{-1} $[BHSO_3MIm]HSO_4$. 0.3 g steroid saponins were mixed with different ratios of solvent to solid and hydrolyzed at 100 °C for 15 min under microwave irradiation.

3.4. Effect of reaction time

Traditionally, diosgenin yield would rise with the increase of microwave reaction time. To investigate the influence of reaction time on the yield of diosgenin, a series of reaction time experiments (5 min, 10 min, 15 min, 20 min, and 25 min) were carried out for comparison. The HPLC analysis results shown in Fig. 5 indicated that the highest yield of diosgenin was obtained after 20 min. If reaction time was longer than 20 min, the diosgenin yield decreased. Moreover, microwave irradiation time was also a significant influencing factor of hydrolysis. The hydrolysis reaction rate at the beginning was much higher than the rates of side reactions. With the increase of microwave irradiation time, the amount of diosgenin also increased. In the late stage of hydrolysis reaction, the rates of side reactions gradually increased with the accumulation of diosgenin, but the main hydrolysis rate decreased due to the consumption of saponins. Diosgenin content reached the maximum value when the side reaction rate was equal to the primary hydrolysis rate. As reaction time further increased, the diosgenin yield decreased. Therefore, microwave irradiation time was set to be 20 min.

In summary, the optimal production conditions for diosgenin are provided as follows: 4 mol L^{-1} $[BHSO_3MIm]HSO_4$, the ratio of solvent to solid of 15:1, the reaction temperature at 100 °C, and reaction time of 20 min. Under the conditions, 29.97 ± 0.51 mg was obtained with 0.3 g steroid saponins.

3.5. Recovering and recycling of ionic liquid

Generally, after the reaction, the reaction solution was filtered to remove some impurities. But some unreacted substrates,

Table 1
Recycling experiments in which new ionic liquid was added to achieve required concentration after every cycle.

Recycling times	Diosgenin content (mg)	Recovery rate of ionic liquid (%)
1	29.97 ± 0.51	92.38
2	29.31 ± 0.52	92.36
3	28.13 ± 0.56	92.33
4	26.80 ± 0.61	92.34
5	25.42 ± 0.58	92.35

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Table 2
Comparison experiment.

Number	Solvent	Consumption (mL)	Heating method	Time (min)	Diosgenin content (mg)	Solvent recyclable
1	Hydrochloric acid	10	Oil bath	300	31.12 ± 0.67	no
2	Hydrochloric acid	4.5	Microwave	20	30.89 ± 0.63	no
3	$[BHSO_3MIm]HSO_4$	4.5	Microwave	20	29.97 ± 0.51	yes

intermediate products, and other water-soluble products remained in the filtrate, thus reducing the effect of ionic liquid in subsequent procedures. Therefore, it is necessary to establish an effective separation method for the recovery of the ionic liquid from the reaction solution. Finally, a convenient recovery method of ionic liquid and unreacted substrates was successfully developed. The filtrate was partitioned by saturated *n*-BuOH twice. Ionic liquid and unreacted saponins were divided into two phases. We could recycle these two important targets simultaneously to avoid waste. *n*-BuOH was also recycled.

The reusability of $[BHSO_3MIm]HSO_4$ ionic liquid was studied to investigate its stability under microwave irradiation. As shown in Table 1, $[BHSO_3MIm]HSO_4$ ionic liquid was stable and effective and the yield was decreased by 15% after five cycles. The results strongly suggested that the acid-functionalized ionic liquid was suitable for multiple hydrolysis of steroid saponins under microwave irradiation.

3.6. Comparison with the conventional method

The selection of hydrolysis methods mainly depend on several important indicators, including feasibility, cleanliness, economy, and safety. We compared acid-functionalized ionic liquid catalytic hydrolysis under optimal conditions with hydrochloric acid hydrolysis. The same sample treatment method and HPLC analysis method were carried out after hydrolysis process. The results were shown in Table 2. The yields of diosgenin obtained by the proposed method, conventional acid hydrolysis method in oil bath, and conventional acid hydrolysis method under microwave irradiation were 29.97 ± 0.51 mg, 31.12 ± 0.67 mg and 30.89 ± 0.63 mg, respectively. The yield of diosgenin obtained by ionic liquid in the microwave method was slightly less than those of other methods. However, the proposed method greatly shortened hydrolysis time and achieved the high efficiency. The proposed method required only 20 min to obtain 96% diosgenin yield, which was obtained after 5 h in traditional method. We also found that the results of hydrochloric acid hydrolysis by different heating modes were basically the same. According to the comparison results, microwave irradiation can not only shorten reaction time, but also reduce solvent consumption. The microwave irradiation plays an important role in the hydrolysis of saponins. In traditional oil bath heating method, the entire volume of solvent was heated unevenly. Heat was passed to the solvent near heat sources firstly and then passed to the distant solvent. This resulted in uneven heating. The whole heating process required long time and more solvent. However, microwave irradiation heated internal dipole molecule and caused high frequency reciprocating motion. Internal solvent and external solvent were heated at the same time. Microwave highlighted the uniformity and quickness. Moreover, any solvent that would pollute the environment was not used in the acid-functionalized ionic liquid catalytic hydrolysis, indicating that it was an efficient and environment-friendly technique.

The material consumptions were shown in Table 3. The positive benefits mainly come from the decrease of reaction time, water use and the reduction of wastewater discharge. The recycling of ionic liquid can also reduce the cost.

Considering the production efficiency and environmental protection, acid-functionalized ionic liquid catalytic hydrolysis under microwave is the better choice for diosgenin production.

Table 3
Material consumptions of traditional and new methods, including 1.5 kg of *Dioscorea zingiberensis* C. H. Wright dry powder in each experiment.

Cost items	Traditional method	New method
Production cost		
Herb (kg)	1.5	1.5
Acid/Ionic liquid	Acid 850 mL	Ionic liquid 160 g [a]
Water (mL)	4250	2250
Time (min)	300	20
Wastewater discharge (mL)	About 4100	–
Income		
Diosgenin (g)	15.6	15.0

[a] The consumption of ionic liquids is calculated based on average five times.

4. Conclusions

The catalytic hydrolysis with an acid-functionalized ionic liquid under microwave irradiation method was successfully developed to convert steroid saponins into diosgenin. The typical 1-sulfobutyl-3-methylimidazolium hydrosulfate ionic liquid ($[BHSO_3MIm]HSO_4$) was used and hydrolysis conditions were optimized. Optimal conditions were provided as follows: 4 mol L^{-1} $[BHSO_3MIm]HSO_4$, the ratio of solvent to solid of 15:1, the reaction temperature at 100 °C, and reaction time of 20 min. Under the optimal conditions, this approach gained the highest yield of diosgenin of 29.97 ± 0.51 mg with 0.3 g steroid saponins. Compared with the regular hydrochloric acid hydrolysis, the proposed approach has the following three advantages. Firstly, the proposed approach obtained approximate yield as the regular hydrochloric acid hydrolysis and reduced 93% reaction time. Secondly, all hydrolysis solvents were environment-friendly and recyclable. Acid-functionalized ionic liquid replaced the traditional hydrochloric acid and greatly alleviated environment pollution. Thirdly, $[BHSO_3MIm]HSO_4$ ionic liquid was stable and effective with only a slight decrease after three cycles. And the unreacted substrates were also recycled by effective recycling method. In summary, this method showed a broad application prospect in diosgenin production.

Acknowledgments

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