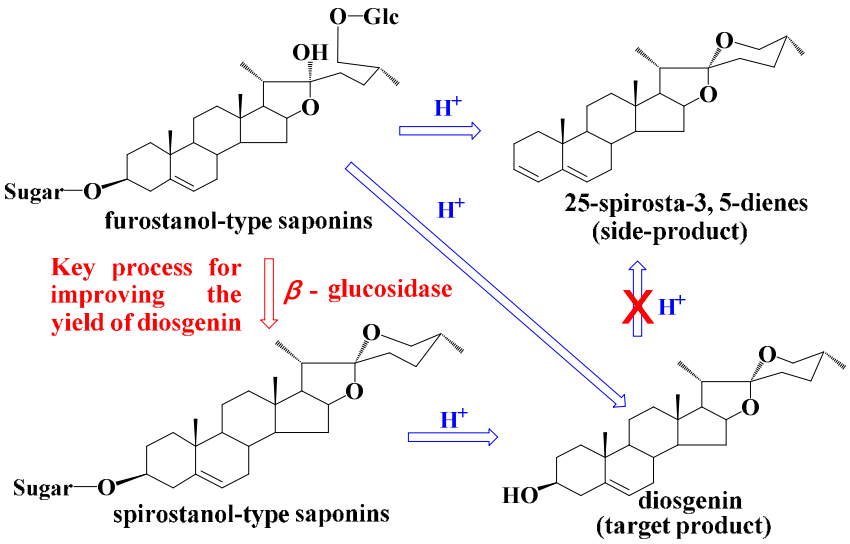
RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Table of Contents entry



Conversion of furostanol saponins into spirostanol saponins avoiding the side-product increases the diosgenin yield by acid hydrolysis of *Dioscorea zingiberensis*.

Conversion of furostanol saponins into spirostanol saponins improves the yield of diosgenin from *Dioscora zingiberensis* by acid hydrolysis

Xu Pang[a, b †], Hong Zhi Huang[a †], Yang Zhao[a], Cheng-Qi Xiong[a], Li Yan Yu[b] and Bai-Ping Ma[a *]

[a] Department of Biotechnology, Beijing Institute of Radiation Medicine, Beijing 100850, China

[b] Institute of Medicinal Biotechnology, Academy of Medical Science & Peking Union Medical College, Beijing 100050, China

† These authors contributed equally to this work.

*Corresponding author: Department of Biotechnology, Beijing Institute of Radiation Medicine. Tel: +86-10-68210077 ext. 930265. E-mail address: mabaiping@sina.com (B. P. Ma)

Abstract:

Current production of diosgenin mainly depends on acid hydrolysis of steroidal saponins from Dioscorea plants, and in China especially the *Dioscorea zingiberensis* C. H. Wright (DZW) was used. The experimental results we obtained demonstrated the furostanol saponins as the main constituents in DZW were prone to generate 25-spirosta-3, 5-diene as side-product during acid hydrolysis process, while the spirostanol saponins hardly generated 25-spirosta-3, 5-diene. The 25-spirosta-3, 5-diene was the key reason leading to the low yield of diosgenin from DZW by acid hydrolysis method. Effective conversion of furostanol saponins into spirostanol saponins can avoid generation of 25-spirosta-3, 5-diene so as to increase the yield of diosgenin, suggesting the importance of this preliminary conversion for improving the yield of diosgenin from DZW by acid hydrolysis. The conversion of furostanol saponins into spirostanol saponins can be done by either enzyme hydrolysis or spontaneous fermentation, while the enzyme hydrolysis is more controllable when compared with spontaneous fermentation.

Keyword:

Dioscorea zingiberensis

Acid hydrolysis

Diosgenin

25-spirosta-3, 5-diene

β-glucosidase

Spontaneous fermentation

1. Introduction

Diosgenin is an important steroidal sapogenin not only because it is used as the starting material for synthesis of steroidal hormones drugs, but also due to its wide range of biochemical and pharmacological activities including reducing the plasma level [1], against skin aging [2], anti-inflammatory [3], antithrombosis [4], anticancer [5] and suppressing acute lung injury [6] etc. At present, the diosgenin all over the world is mainly provided by China and Mexico. In China, *Dioscorea zingiberensis* C. H. Wright (DZW), a particular and resourceful cultivated plant especially in the areas of Shiyan and Enshi in Hubei province and Ankang in Shaanxi Province, is always used as feedstock for production of diosgenin. The conventional method for production of diosgenin is acid hydrolysis of steroidal saponins from Dioscorea plants, generally using sulfuric acid [7]. However, the acid treatment inevitably leads to a severe environmental pollution problem [8-9]. Efforts on application of biotechnology instead of acid treatment on production of diosgenin were reported [10-13], while no method without acid treatment has been industrialized so far and diosgenin is still produced by the acid hydrolysis method. Nevertheless, if the key factors to improve the yield of diosgenin based on the present production process could be figured out, the environmental pollution could be mitigated.

In this study, it was found that the yield of diosgenin from DZW by direct acid hydrolysis was very low, and much side-product was observed in the acid hydrolyzate. The side-product identified as 25-spirosta-3, 5-diene was a steroid derivative supposed to be from the starting steroidal saponins from DZW. This motivated us, and finally we clearly proved that the furostanol saponins taking a high level content in DZW were prone to generate 25-spirosta-3, 5-diene in acid hydrolysis process, while the spirostanol saponins hardly generated it. Therefore, it was considered that 25-spirosta-3, 5-diene mainly derived from the furostanol saponins during the acid hydrolysis reaction might be the key reason leading to the low yield of diosgenin, and effective conversion of furostanol saponins into spirostanol saponins can improve the yield of diosgenin from DZW. Consequently, by pretreatment with β-glucosidase, the furostanol saponins of DZW were totally converted into spirostanol saponins, and the yield of diosgenin from DZW by acid hydrolysis was notably enhanced, suggesting the importance of this preliminary conversion for improving the yield of diosgenin from DZW by acid hydrolysis.

2. Results and discussion

2.1. 25-spirosta-3, 5-diene as side-product leading to low yield of diosgenin by direct acid hydrolysis.

Conventional method for diosgenin production is direct acid hydrolysis of steroidal saponins from Dioscorea plants. According to conventional method, the yield of diosgenin from DZW (heat-dried DZW, the same as follows) by direct acid hydrolysis (3 mol·L^{-1} sulfuric acid at 95 °C for 5 hours) was determined to be 1.26% in our laboratory, which is much low. In order to know the reason why the yield of diosgenin from DZW by conventional acid hydrolysis was so low, the hydrolyzate was subjected to HPLC-ELSD analysis. As the Fig. 1A showed, the diosgenin was not the only product, meanwhile, much side-product identified as 25-spirosta-3, 5-diene existed in the hydrolyzate and the ratio of 25-spirosta-3, 5 diene and diosgenin obtained according to their HPLC-ELSD peak area was 1.05:1, suggesting that 25-spirosta-3, 5-diene generated in the acid hydrolysis process might be the key reason leading to the low yield of diosgenin.

2.2. 25-spirosta-3, 5-diene derives from the starting furostanol saponins during acid hydrolysis.

Six typical compounds (the structures were drawn in Fig. 2) in the DZW, including two furostanol saponins, namely parvifloside and deltoside, three spirostanol saponins, namely zingiberensis newsaponin, deltonin and prosapogenin A of dioscin, and the sapogenin diosgenin were subjected to acid hydrolysis reactions under typical conditions, namely in 3 mol·L^{-1} sulfuric acid at 95 °C for 5 hours, before their hydrolyzates were analyzed by HPLC-ELSD. Based on HPLC-ELSD profiles of acid hydrolyzates from the six compounds above, it was found that a large amount of 25-spirosta-3, 5 diene generated from parvifloside and deltoside (Fig. 3A and 3B), and the zingiberensis newsaponin, deltonin and prosapogenin A of dioscin were mostly converted to diosgenin with little 25-spirosta-3, 5 diene (Fig. 3C, 3D and 3E). In addition, it was found that diosgenin was hardly converted to 25-spirosta-3, 5 diene under these reaction conditions (Fig. 3F), which is in contrast to a previous report stating that 25-spirosta-3, 5 diene derived from diosgenin by dehydration [15]. Ratios of 25-spirosta-3, 5 diene to diosgenin produced from six compounds were in table 1.

Furthermore, the effect of acid concentrations on the yield of 25-spirosta-3, 5 diene was studied based on HPLC-ELSD analysis of acid hydrolyzates from parvifloside and deltoside under different acid concentrations (Fig. S1). A portion of parvifloside and deltoside were transformed into 25-spirosta-3, 5 diene even at 0.5 mol·L^{-1} sulfuric acid, suggesting that the conversion of furostanol saponins into 25-spirosta-3, 5 diene during acid hydrolysis reaction is inevitable. The yields of 25-spirosta-3, 5 diene from parvifloside and deltoside in the 0.5, 1 and 2 mol·L^{-1} sulfuric acid solutions were lower than that in 3 mol·L^{-1} sulfuric acid. However, our previous study has proved that the sulfuric acid of concentration less than 2.5 mol·L^{-1} converted total saponins into diosgenin very limitedly (data not shown). That was the reason why 3 mol·L^{-1} sulfuric acid was finally used in this work. The results clearly indicated that 25-spirosta-3, 5 diene mainly derives from the furostanol saponins during the acid hydrolysis process.

2.3. Proposed reaction pathways of furostanol and spirostanol saponins during acid hydrolysis.

Fig. 4 presented the possible reaction pathways of furostanol and spirostanol saponins during acid hydrolysis process. To furostanol saponins, after protonation on the oxygen atom at C-3 and C-26, a portion of intermediate A generated intermediate B by the cleavage between the sugar moiety and oxygen atom at C-3, and then intermediate B generated diosgenin by dehydration reaction. Meanwhile, by the cleavage between the oxygen atom and carbon atom of C-3, another portion of intermediate A generated intermediate C, and then intermediate D derived from intermediate C by charge transfer. Furthermore, intermediate D lost the hydrogen atom at C-3 and afforded intermediate E, and finally intermediate E yielded 25-spirosta-3, 5 diene by dehydration reaction. However, to sapirostanol saponins, after protonation on the oxygen atom at C-3, almost all the intermediate F were converted into diosgenin by cleavage between the oxygen atom and carbon atom of C-3.

2.4. Conversion of furostanol saponins into spirostanol saponins can avoid 25-spirosta-3, 5 diene and increase the yield of diosgenin.

It has thus been proved that 25-spirosta-3, 5 diene mainly derives from the furostanol saponins during acid hydrolysis process. Therefore, effective conversion of furostanol saponins into

spirostanol saponins before acid hydrolysis is important to obtain high yield of diosgenin from DZW. Enzymatic treatment is an effective and controllable method for bioconversion, and *β*-glucosidase is most efficient to hydrolyze furostanol saponins into spirostanol saponins. Accordingly, the *β*-glucosidase isolated from *Aspergillus flavus* in our lab was used to treat DZW. HPLC-ELSD profiles of 70% ethanol extract from DZW and *β*-glucosidase treated DZW are shown in Fig.5. The parvifloside and deltoside were the main constituents of DZW (Fig. 5A), and in *β*-glucosidase treated DZW, all of them were converted into prosapogenin A of dioscin (Fig. 4B). The yield of diosgenin after acid hydrolysis of *β*-glucosidase pretreated DZW was enhanced to 2.82%, far more than that by direct acid hydrolysis. Moreover, HPLC-ELSD profile of the acid hydrolyzate from *β*-glucosidase pretreated DZW presented that, except for diosgenin, there was almost no 25-spirosta-3, 5 diene was observed (Fig. 1B), clearly proving that 25-spirosta-3, 5 diene was indeed the key factor leading to the low yield of diosgenin.

2.5. Conversion of furostanol saponins into spirostanol saponins by spontaneous fermentation.

Literatures reported that the pretreatment of DZW by spontaneous fermentation before acid hydrolysis can improve the yield of diosgenin [16, 17]. In Ankang, Shaanxi province of China, the method of spontaneous fermentation was actually applied in diosgenin production factory. A previous study reported that the furostanol saponins can be hydrolyzed to the corresponding spirostanol saponins by endogenous glucosidase obtained from the same plant [18], which suggested that, by spontaneous fermentation, the furostanol saponins of DZW can be converted into spirostanol saponins by endogenous glucosidase, leading to improve the yield of diosgenin.

According to the common method in literatures, a heat-dried DZW sample was subjected to spontaneous fermentation experiment, and the yield of diosgenin by acid hydrolysis was finally determined to be 1.40% (experimental details see supporting information), and there is no notable improvement compared with the yield of 1.26% by direct acid hydrolysis. HPLC-ELSD analysis showed that parvifloside and deltoside were hardly converted into the spirostanol saponins by spontaneous fermentation (Fig. S2), and in the acid hydrolyzate, much 25-spirosta-3, 5 diene still existed (Fig. S3). Generally, the fresh DZW is always dried in air or by heating after collection. In order to better understand the conversion efficiency of spontaneous fermentation on DZWs under

different conditions, the chemical constituents of the fresh DZW, air-dried DZW and heat-dried DZW, as well as their spontaneous fermented residues were compared by HPLC-ELSD (experimental details see supporting information). As shown in Fig. 6, among the three kinds of DZW, the main constituents were the furostanol saponins, namely parvifloside and deltoside. After spontaneous fermentation, the parvifloside and deltoside in fresh DZW were totally converted into corresponding spirostanol saponins, namely zingiberensis newsaponin and deltonin (Fig. 7A), while little zingiberensis newsaponin and deltonin were detected in hydrolyzed air-dried and heat-dried DZWs (Fig. 7B and 7C), suggesting that the endogenous glucosidase in air-dried and heat-dried DZWs might be inactivated during the drying process, so that the furostanol saponins cannot be effectively converted. These results indicated that conversion of DZW furostanol saponins into spirostanol saponins could be conducted by spontaneous fermentation, but the conversion efficiency was influenced by the activity of endogenous glucosidase in raw material.

3. Experimental

3.1. Materials

The fresh tubers of *Dioscorea zingiberensis* C. H. Wright were collected from Ankang, Shanxi province, China. Part of fresh DZW tubers were stored at -20 °C for keeping fresh (fresh DZW), and the others were dried in oven at 80 °C (heat-dried DZW) and dried in air at room temperature (air-dried DZW) to a constant weight, respectively. The *β*-glucosidase was isolated from *Aspergillus flavus* in our lab previously [14]. Pure diosgenin, 25-spirosta-3, 5-diene, parvifloside, deltoside, zingiberensis newsaponin, deltonin, prosapogenin A of dioscin were all obtained from DZW tubes and their acid hydrolyzate in our laboratory (purity > 95% detected by HPLC-ELSD), and identified by NMR and MS experiments (Fig. 2). All chromatographic grade solvents used were purchased from Fisher Scientific Co. Ltd, and solvents with analytical grades were all purchased from Beijing chemical plant.

3.2. General procedures

Acid hydrolysis of DZW was carried out by depositing sample in flask with 3 $mol \cdot L^{-1}$ sulfuric acid and kept at 95 °C for 5 hours under the condition of reflux. Acid hydrolysis of compounds was performed by keeping each compound in airtight test tube with sulfuric acid at 95°C for 5 hours. Enzyme hydrolysis of DZW was performed by depositing raw DZW mixed with water and *β*-glucosidase in airtight triangular flask and incubated at 35 °C for 30 hours. Spontaneous fermentation was carried out by incubating raw DZW mixed with water at 35 °C for 30 hours. All chemical analysis were performed by HPLC-ELSD which were carried out by Waters 2695 system equipped with a PL-ELS 2000 evaporative light scattering detector (Temp: 90 °C, Gas: 1.6 L/min) and Phecda C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, ODS).

3.3. Determination of the yield of diosgenin from DZW and chemical characterization of the acid hydrolyzate

5 g heat-dried DZW was smashed, and then mixed with 3 $mol \cdot L^{-1}$ sulfuric acid (100 mL), followed by heating at 95 °C for 5 hours under reflux. After neutralized with NaOH and filtrated, the residue (together with filter paper) was dried at 80 °C and extracted with ethyl acetate at 85 °C

under reflux for three times (100 mL, 70 mL, 50 mL, 1 h each time). When the ethyl acetate was recovered, the residue was dissolved in 50 mL methanol and subjected to HPLC-ELSD analysis with the mobile phase of acetonitrile-water (94:6, v/v) to determine the yield of diosgenin. Meanwhile, the hydrolysate was analyzed by HPLC-ELSD with the gradient mobile phase consisting of menthol-water (0.00-14.00-14.01-35.00 min, 97%-97%-100%-100% menthol).

3.4. Acid hydrolysis of typical compounds in DZW and chemical characterization

Parvifloside, deltoside, zingiberensis newsaponin, deltonin, prosapogenin A of dioscin and diosgenin (each 5 mg) were put into the airtight test tube with 3 mL of 3 $mol \cdot L^{-1}$ sulfuric acid and heated at 95 °C for 5 hours, respectively. After neutralized with NaOH, each hydrolyzate was extracted with ethyl acetate (3 mL). Until the ethyl acetate layer was evaporated completely, the residues were dissolved with methanol and subjected to HPLC-ELSD analysis with the gradient mobile phase consisting of menthol-water (0.00-14.00-14.01-35.00min, 97%-97%-100%-100% menthol). Additionally, in order to study the effect of acid concentrations on the yields of diosgenin and 25-spirosta-3, 5 diene from furostanol saponins of DZW, the parvifloside and deltoside were hydrolyzed in sulfuric acid at the concentrations of 0.5 $mol \cdot L^{-1}$, 1.0 $mol \cdot L^{-1}$, and 2.0 $mol \cdot L^{-1}$ sulfuric acid. 15 mg of parvifloside was dissolved in 5 mL of 0.5 $mol \cdot L^{-1}$, 1 $mol \cdot L^{-1}$, 2 $mol \cdot L^{-1}$, and 3 $mol \cdot L^{-1}$ sulfuric acid, respectively, and then heated at 95 °C for 5 hours. After neutralized with NaOH, the hydrolyzates were extracted with ethyl acetate (3 mL), respectively. After the ethyl acetate was evaporated completely, each residue was dissolved in methanol for HPLC analysis. Likewise, the deltoside was treated using the same condition as parvifloside. The chemical characterization of acid hydrolyzates from the compounds above was performed using HPLC-ELSD with the gradient mobile phase consisting of menthol-water: (0.00-14.00-14.01-35.00 min, 97%-97%-100%-100% menthol).

3.5. Determination of the yield of diosgenin from *β*-glucosidase treated DZW and chemical characterization of the acid hydrolyzate

First, 5 g smashed heat-dried DZW mixed with 30 mL water and 89 mg *β*-glucosidase (based on the enzyme protein amount, the same as follows) were incubated at 35 °C for 30 hours to obtain the pretreated DZW sample. Then, the treated DZW was added to 70 ml of 3 $mol \cdot L^{-1}$

sulfuric acid and additional 6 mL of 18 mol·L^{-1} M sulfuric acid (finally 3 mol·L^{-1} sulfuric acid), and then heated under reflux at 95 °C for 5 hours. After neutralization with NaOH and flirtation, the residue (together with filter paper) was dried at 80 °C and extracted with ethyl acetate at 85 °C under reflux for three times (100 mL, 70 mL, 50 mL, 1 h each time). When the ethyl acetate was recovered, the residue was dissolved in 50 mL methanol and subjected to HPLC-ELSD with the mobile phase of acetonitrile-water (94:6, v/v). Chemical characterization of acid hydrolyzate from *β*-glucosidase treated DZW was performed using HPLC-ELSD with the gradient mobile phase consisting of menthol-water (0.00-14.00-14.01-35.00 min, 97%-97%-100%-100% menthol).

3.6. Chemical characterization of DZW and *β*-glucosidase treated DZW

The heat-dried DZW and *β*-glucosidase treated DZW (sample preparation refers to above part) were extracted with 70% EtOH under reflux at 95 °C for 1 hour, respectively, and then the extracts were subjected to HPLC-ELSD analysis. An acetonitrile-water solution (gradient, 0.00-17.00-19.00-20.00-25.00-25.01-35.00-40.00 min, 27%-27%-35%-45%-45%-66%-66%-90% acetonitrile) was used as mobile phase.

3.7. Chemical characterization of fresh, air-dried and heat-dried DZWs, together with their spontaneous fermented residues

The fresh, air-dried and heat-dried DZWs were extracted with 70% EtOH at 95 °C for 1 hour, respectively. After filtration, the extracts were subjected to HPLC-ELSD analysis directly. For chemical characterization of their spontaneous fermented residues, the pounded fresh DZW, the crushed air-dried and heat-dried DZWs were mixed with 30 mL water and incubated at 35 °C for 30 hours, respectively. Then each fermented residue was extracted with 70% EtOH at 95 °C for 1 hour. After filtration, the extracts were also subjected to HPLC analysis. HPLC analysis was performed using HPLC-ELSD with the gradient mobile phase consisting of acetonitrile-water (0.00-17.00-19.00-20.00-25.00-25.01-35.00-40.00 min, (27%-27%-35%-45%-45%-66%-66%-90% acetonitrile).

Conclusion

For the first time, we demonstrated that the importance of the preliminary conversion of furostanol saponins into spirostanol saponins for improving the yield of diosgenin from DZW by acid hydrolysis. The effective conversion of these furostanol saponins into spirostanol saponins can avoid the generation of 25-spirosta-3, 5 diene so as to increase the yield of diosgenin. Conversion of DZW furostanol saponins into spirostanol saponins can be conducted by enzyme (such as *β*-glucosidase) hydrolysis or spontaneous fermentation. Spontaneous fermentation is a practical and economic conversion method to produce diosgenin, while its conversion efficiency is not well controllable due to the variables of endogenous glucosidase in raw DZWs. The enzyme hydrolysis, when compared with the spontaneous fermentation, is more effective and controllable, so that more suitable for the industrialization of production of diosgenin.

Notes and references

1 H. Chen, C. Wang, C. T. Chang, *Nutrition*, 2003, 19, 646-651.

2 Y. Tada, N. Kanda, A. Haratake, M. Tobiishi, H. Uchiwa, S. Watanabe, *Steroids*, 2009, 74, 504-511.

3 C. H. Huang, C. Y. Ku, T. R. Jan, *Planta Med*., 2009, 75, 1300-1305.

4 G. H. Gong, Y. Qin, W. Huang, *Phytomedicine*, 2010, 18, 458-463.

5 S. Rajput, M. Mandal, Eur. *J Cancer Prev.*, 2012, 21, 205-215.

6 M. Gao, L. Chen, H. Yu, Q. Sun, J. Kou, B. Yu, *Int. Immunopharmacol*., 2013, 15, 240-245.

7 J. W. Rothrock, P. A. Hammes, W. J. Mcaleer, *Ind. Eng. Chem*., 1957, 49, 186-188.

8 H. Z. Zhao, P. Cheng, B. Zhao, J. R. Ni. *Process Biochem*., 2008, 43, 1427-1431.

9 P. Cheng, H. Z. Zhao, B. Zhao, J. R. Ni. *Bioresour. Technol*., 2009, 100, 2918-2915.

10 L. Liu, Y. S. Dong, S. S. Qi, H. Wang, Z. L. Xiu, *Appl. Microbiol. Biotechnol*., 2010, 85, 933-940.

11 W. Liu, W. Huang, W. Sun, Y. Zhu, J. Ni, *World J Microbiol. Biotechnol*., 2010, 26, 1171-1180.

12 J. Lei, H. Niu, T. Li, W. Huang, *World J Microbiol. Biotechnol*., 2012, 28, 1309-1314.

13 M. Wei, Y. Bai, M. Ao, W. Jin, P. Yu, M. Zhu, L. Yu, *Bioresour. Technol*., 2013, 146, 549-555.

14 H. Z Huang, M. Zhao, L. Lu, D. W. Tan, W. B. Zhou, C. Q. Xiong, Y. Zhao, X. B. Song, L. Y. Yu, B. P. Ma, *J Mol. Catal. B-Enzym*., 2013, 98: 1-7.

15 Y. E. Peng, Z. H. Yang, Y. X. Wang, Z. Y. Liu, J. G. Bao, Y. Hong, *Chem. Eng. Res. Des.* 2011, 89, 2620-2625.

16 X. L. Tang, Z. L. Xu, B. Xia, Y. Y. Shi, *J Plant. Resour. Environ*., 2004, 13, 35-37.

17 Z. J. Qiao, X. M. Li, *Acta Bot. Boreal. Occident. Sin.*, 2006, 15, 212-215.

18 K. Inoue, Y. Ebizuka, *FEBS Letters*, 1996, 378, 157-160.

Legends for Figures

Fig.1 HPLC-ELSD profiles of acid hydrolyzates from DZW (A) and *β*-glucosidase treated DZW (B) (The DZW samples were obtained by heating fresh DZW at 80 °C).

Fig.2 Structures of diosgenin (**1**), 25-spirosta-3, 5-dienes (**2**), parvifloside (**3**), deltoside (**4**), zingiberensis newsaponin (**5**), deltonin (**6**) and prosapogenin A of dioscin (**7**).

Fig.3 HPLC-ELSD chemical profiles of hydrolyzates from parvifloside (A), deltoside (B), zingiberensis newsaponin (C), deltonin (D), prosapogenin A of dioscin (E) and diosgenin (F) by acid hydrolysis in 3 $mol \cdot L^{-1}$ sulfuric acid.

Fig.4 Main proposed reaction pathways of furostanol saponins and spirostanol saponins during acid hydrolysis process.

Fig.5 HPLC-ELSD profiles of DZW (A) and *β*-glucosidase treated DZW (B) (The DZW samples were obtained by heating fresh DZW at 80 °C).

Fig.6 HPLC-ELSD profiles of the fresh DZW (A), air-dried DZW (B) and heat-dried DZW (C).

Fig.7 HPLC-ELSD profiles of the hydrolyzed fresh DZW (A), hydrolyzed air-dried (B) and hydrolyzed heat-dried DZW (C).

Table 1 Ratios of 25-spirosta-3, 5 diene to diosgenin obtained by HPLC-ELSD from parvifloside, deltoside, zingiberensis newsaponin, deltonin, prosapogenin A of dioscin and diosgenin by acid hydrolysis with 3 $mol \cdot L^{-1}$ sulfuric acid.

Compound	**Peak area ratio (%) (25-spirosta-3,5 diene : diosgenin)**
Parvifloside	127.77
Deltoside	115.05
Zingiberensis Newsaponin	3.63
Deltonin	1.83
Prosapogenin A of dioscin	0.40
Diosgenin	0

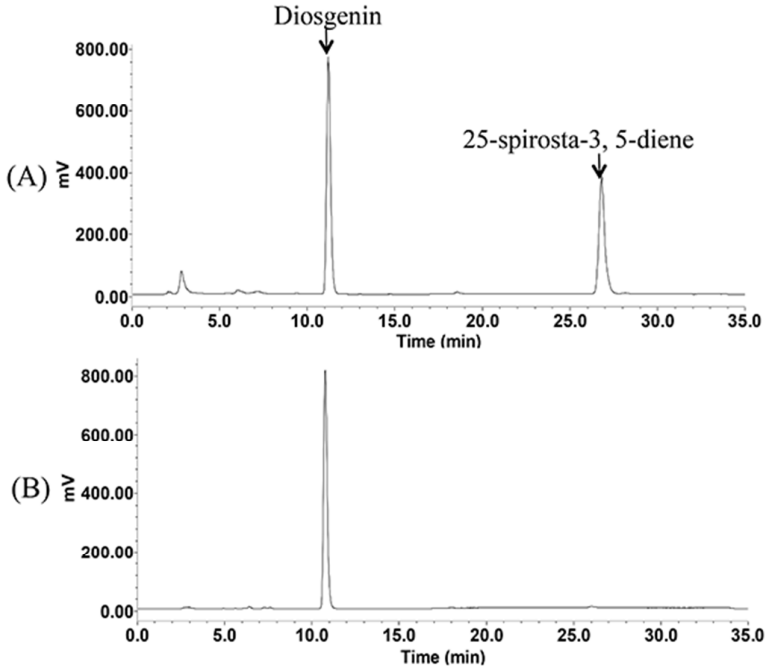


Fig.1 HPLC-ELSD profiles of acid hydrolyzates from DZW (A) and *β*-glucosidase treated DZW (B) (The DZW samples were obtained by heating fresh DZW at 80 °C).

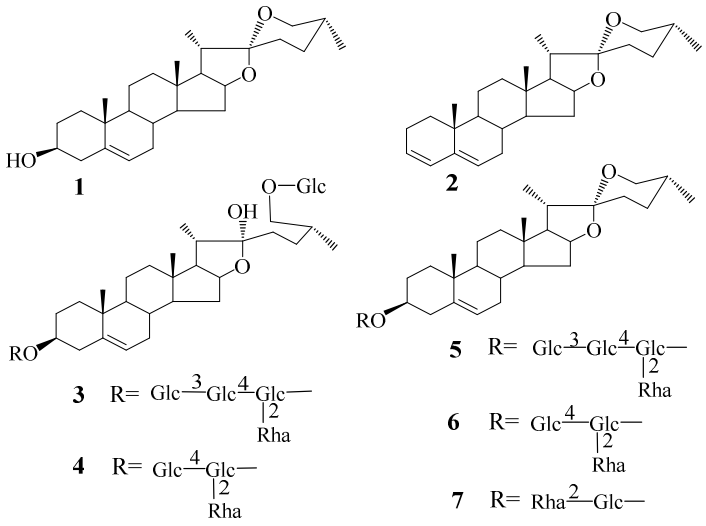


Fig.2 Structures of diosgenin (**1**), 25-spirosta-3, 5-diene (**2**), parvifloside (**3**), deltoside (**4**), zingiberensis newsaponin (**5**), deltonin (**6**) and prosapogenin A of dioscin (**7**).

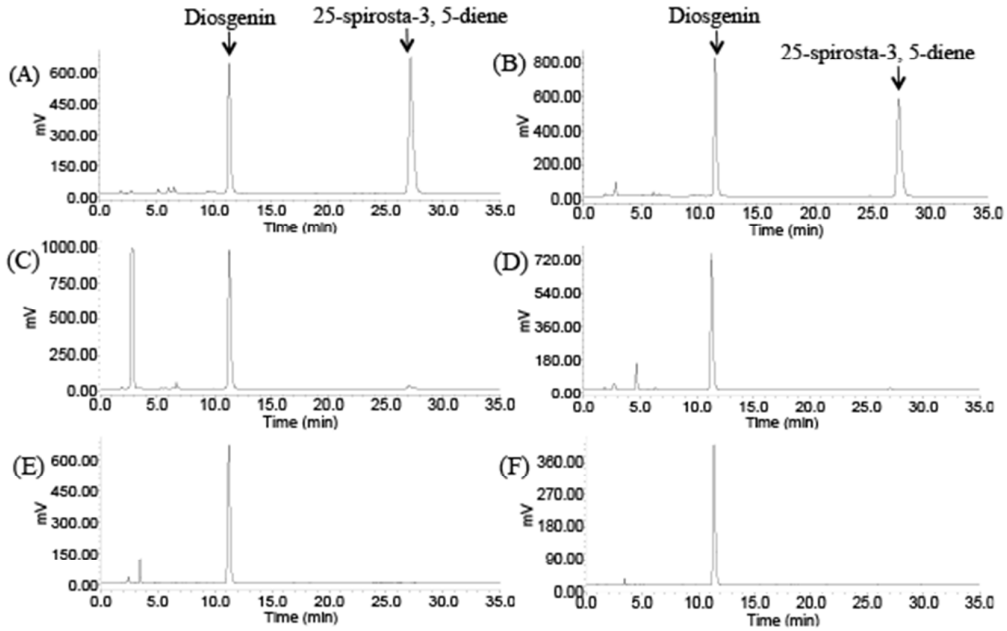


Fig.3 HPLC-ELSD chemical profiles of hydrolyzates from parvifloside (A), deltoside (B), zingiberensis newsaponin (C), deltonin (D), prosapogenin A of dioscin (E) and diosgenin (F) by acid hydrolysis in 3 mol·L^{-1} sulfuric acid.

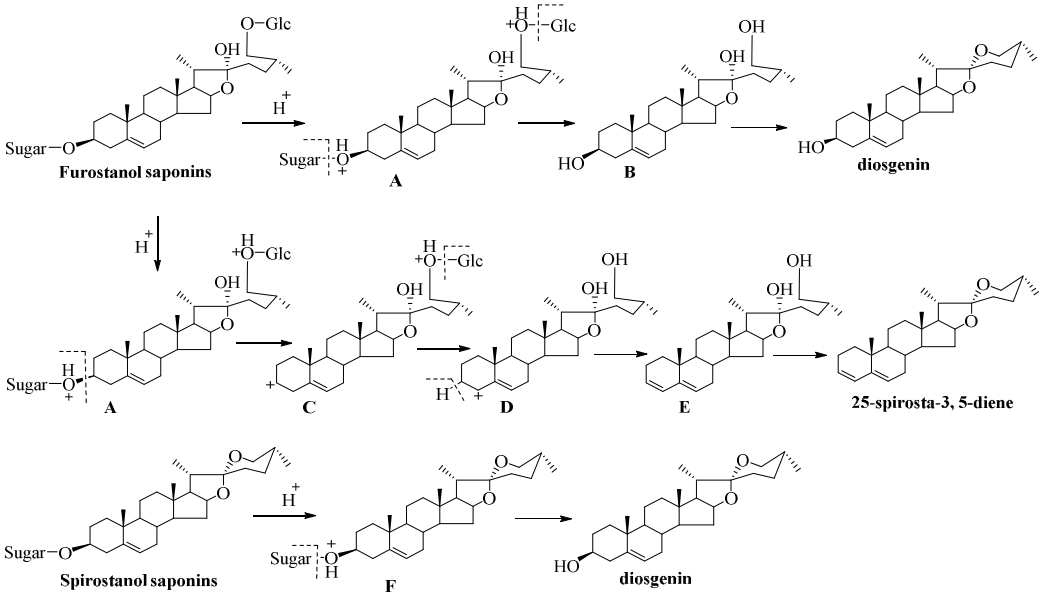


Fig.4 Main proposed reaction pathways of furostanol saponins and spirostanol saponins during acid hydrolysis process.

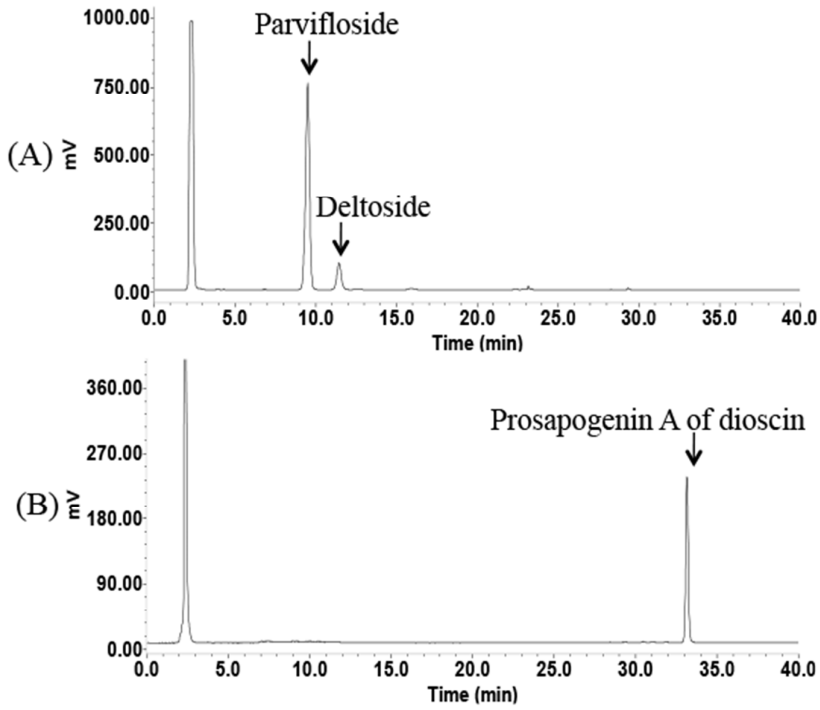


Fig.5 HPLC-ELSD profiles of DZW (A) and *β*-glucosidase treated DZW (B) (The DZW samples were obtained by heating fresh DZW at 80 °C).

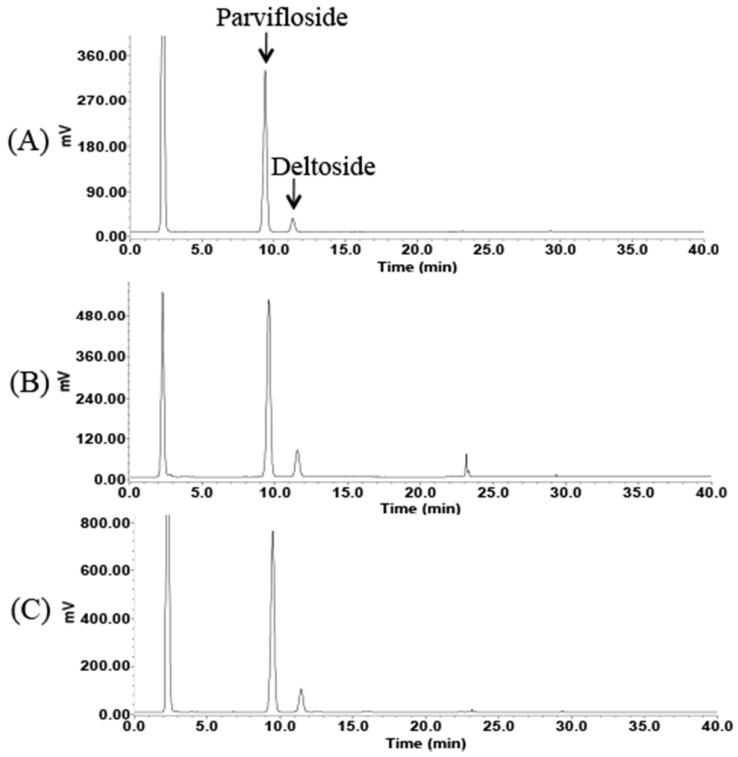


Fig.6 HPLC-ELSD profiles of the fresh DZW (A), air-dried DZW (B) and heat-dried DZW (C).

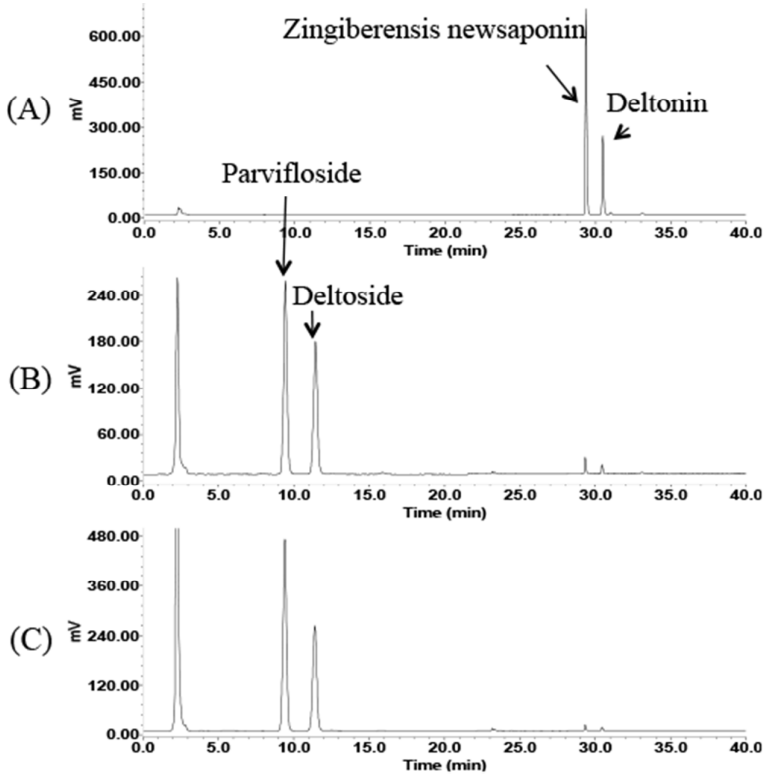


Fig.7 HPLC-ELSD profiles of the hydrolyzed fresh DZW (A), hydrolyzed air-dried (B) and hydrolyzed heat-dried DZW (C).