



## Research Paper

# Physical and chemical analysis of diosgenins prepared by water separation and high temperature and pressure hydrolysis from different kinds of *Dioscorea* rhizomes

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### ABSTRACT

Rhizome of *Dioscorea nipponica* Makino, *Dioscorea composita* and *Dioscorea zingiberensis* (containing wild and planting) were taken as raw materials for diosgenin production. Four kinds of diosgenin products were prepared through saponin pulp isolated by water separation, fibre and starch separated as a subsidiary to recycle resources, high temperature and pressure hydrolysis of saponin pulp, and the extraction rate of diosgenin was more than 94%. Four kinds of diosgenin products were white, and the microstructures were all blocky according to the results of SEM. Then they were analyzed by XRD, XRD, FT-IR, HPLC and melting point detection. Thus the results showed that peaks of four kinds of diosgenin products almost overlap, the purity were all more than 95%, and the melting points were all higher than 195°C. The four kinds of diosgenin products accord with the standard of industry from the physical and chemical testing and analysis.

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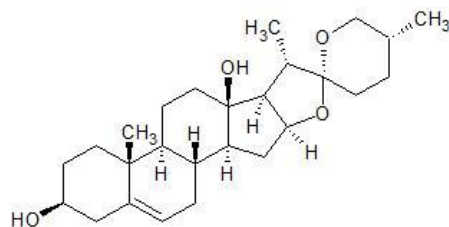
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**Key words:** Dioscorea, saponin, diosgenin, preparation, physical and chemical analysis

## INTRODUCTION

There are about 10 genera and 650 species of *Dioscorea* in the world, which are widely distributed in temperate and tropical regions, but only about 80 species are distributed in China, among which *Dioscorea nipponica* Makino, *Dioscorea composita* and *Dioscorea zingiberensis* are the main raw materials for extracting saponin and preparing diosgenin (National Pharmacopoeia Commission, 2010; Xu et al, 1996). Diosgenin has a lot of pharmacological activities such as cough relief, desensitization, cholesterol lower, and stimulation of the liver bile secretory cell growth (Pan et al, 2013). Besides, diosgenin called the "mother of the hormone" is a starting material and intermediate of the basis of the synthetic steroid hormone drugs, which can compound about 200 kinds of drugs such as adrenal cortical hormone, sex hormone, anabolic hormones etc. (the

formula as shown in **Figure 1**) (Wang et al, 2011). According to data statistics, the annual sales volume of steroidal drugs in the global market has exceeded \$40 billion at present, accounting for about 10% of the world's total medical products. China is the leading international supplier of diosgenin and its intermediate, and the annual output of diosgenin reaches 5,000 tons (Jin et al, 2017). The preparation of diosgenin underwent Rothrok method (firstly hydrolyzed and then extracted) to Marker method (firstly extracted and then hydrolyzed) (Zhang et al, 2017). Major researches on diosgenin mainly focus on the pharmacological analysis and downstream drug synthesis in recent years, and ignore the development of green process of diosgenin preparation. The diosgenin preparation technology of double, surface activity, trasonic and



**Figure 1:** Structure of diosgenin.



**Figure 2:** Four kinds of dioscorea rhizomes (a DNM, b DC, c WDZ, d PDZ).

microwave assisted extraction were only processing laboratory stage, which have many shortcomings such as high cost, turmeric pigment removal, difficult to industrial mass production, etc.. In China, most enterprises use 70% ethanol extraction process of diosgenin at present, which not only the preparation cost is high due to the use of organic solvents, but also the problem of yellow pigment hard to be moved plague enterprises (Linhong et al, 2004). So traditional acid hydrolysis process must be used when the *D. zingiberensis* is as raw material, but traditional acid hydrolysis process is not environmentally required to operate, and forbidden now. In this study, firstly, saponins from *D. nipponica Makino*, *D. composita*, wild and cultivated *D. zingiberensis* as the materials were isolated from pulp by water separation and secondly, separated the fibre and starch as a subsidiary to recycle resources, and then to hydrolysis in high temperature and high pressure. Diosgenin was obtained by extraction at last. Through testing and analysis, the four types of diosgenin products accord with the industry standard.

## MATERIALS AND METHODS

Four of dioscorea rhizomes of *D. nipponica Makino*, *D. composita*, wild *D. zingiberensis* and cultivated *D. zingiberensis* (hereinafter referred to DNM, DC, WDZ, PDZ respectively) as raw materials are shown in **Figure 2**. They were provided by guangdong yangjiang biotechnology co., LTD, detected by Guo Gao (engineer).

## Reagents

Diosgenin standard (China food and drug testing institute, batch no.: 11539-200001); Acetonitrile (chromatography, Shanghai sincere fine chemical co., LTD.), methanol and acetic acid glacial (analytical pure, Tianjin Jinyue fine chemical co., LTD.), hydrochloric acid (analytical pure, Huizhou Xiangsheng chemical co., LTD.), petroleum ether (60 ~ 90°C) (Huizhou Xiangsheng chemical co., LTD.), perchlorate and vanilla aldehyde (analytical pure, Shanghai

fondle born industrial co., LTD.), ultrapure water (laboratory homemade).

## Instruments

High performance liquid chromatography (HPLC) instrument (LC-20AT, Waters company, USA), scanning electron microscope (JSM-6360LV, JEOL company, Japan), Fourier transform infrared spectrometer (IR Affinity, Shimadzu company, Japan), ultraviolet/visible spectrophotometer (UV-9600, Beijing Rayleigh analytical instruments co., LTD., China), X-ray diffractometer (Empyrean, panak company, Nether company, China ), synchronous thermal analyzer (STA449F3, Natch company, Germany), electronic balance (FA/JA, Shanghai jing company, China ).

## Diosgenin preparation method

### Technological process

Dioscorea rhizome → soaking → breaking the wall → separating fiber and starch → enriching and concentrating saponin → hydrolyzing saponin pulp → filtering, washing and drying → extracting diosgenin → obtaining diosgenin product.

### Experiment step

Rhizomes (100 g) was soaked for 4 h, taken into a wall breaking machine, added water (400 mL), broken for 40 s, and filtered with 200-mesh sieve. Residues were washed 3 times, washing liquid and filtrate were combined, and saponin pulp was enriched and concentrated. The acid concentration of saponin pulp was adjusted to  $1 \text{ mol} \cdot \text{L}^{-1}$  with sulfuric acid. Then, the saponin pulp was placed in the high temperature and high pressure reaction kettle, stirred continuously under specific pressure, and kept heating at  $130^\circ\text{C}$  for 60 min. The hydrolyzed pulp was filtered and washed to neutral, dried at  $105^\circ\text{C}$ , packaged and extracted by petroleum ether at  $80^\circ\text{C}$  for 6h, and the crystal diosgenin was obtained.

## Detection method

### Thermal analysis method

The temperature and heat coordinates were calibrated with high purity indium, and the whole process was conducted under drying. The purge air flow rate was  $20 \text{ mL/min}$ . Dioscorea saponin samples ( $5.0 \pm 0.1$ ) mg were accurately weighed, put into aluminum crucible whose lid with holes in the center, and used the same empty crucible as the reference. Then heating rate of  $10^\circ\text{C}$  was set respectively,

heated to  $600^\circ\text{C}$ , and kept the temperature for 5 min (Feixiong, 2014).

## Spectrophotometric detection of diosgenin yield

(1) Wavelength scanning: Dried diosgenin standard 4.3 mg was taken into 25 mL flask, volume with methanol and mixed well (Kang et al, 2012). The maximum absorption wavelength was determined by scanning in the range of 400-600 nm on the UV spectrophotometer, and the maximum absorption wavelength was obtained at 457 nm.

(2) Set up the standard curve: Firstly, 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 ml standard solution were accurately absorbed in a 10 ml covered test tube, respectively, and the solvent was removed in the water bath at  $80^\circ\text{C}$ . Then 0.2 mL 5% vanillin glacial acetic acid solution and 0.8 mL perchloric acid were added, shaken well, and taken into water bath at  $70^\circ\text{C}$  for 15 min. After cooling with ice water, glacial acetic acid was added to a volume of 10 mL, and the test tube was covered and placed for 30 min. The absorbance was measured at the maximum absorption wavelength with the reagent as reference. According to the standard curve of absorbance value of each concentration, the regression equation is  $y=21.022x-0.0078$  ( $R^2=0.9954$ ). The results showed that diosgenin had a good linear relationship in the range of  $1.72 \times 10^{-3}$  to  $18.92 \times 10^{-3} \text{ g} \cdot \text{L}^{-1}$ .

## HPLC detection of diosgenin purity

(1). Chromatographic and determination conditions: Octylsilane bonded silica gel was the filler, acetonitrile -- water (90:10) was the mobile phase, flow rate was  $1 \text{ mL} \cdot \text{min}^{-1}$ , detection wavelength was 208 nm, column temperature was  $30^\circ\text{C}$  (Wang, 2009).

(2). Preparation of reference solution: Diosgenin standard 10 mg was placed in 25 mL flask, dissolved with methanol, and diosgenin concentration was  $0.4 \text{ mg} \cdot \text{mL}^{-1}$ .

(3). Preparation of the sample solution: Diosgenin product 5 mg was placed in a 25 mL flask, dissolved with methanol, and diosgenin concentration was  $0.2 \text{ mg} \cdot \text{mL}^{-1}$ .

(4). Investigation of linear relationship. 0.5, 1.25, 2.5, 3.75, 5 and 6.25 mL of the reference solution were placed in a 10 ml flask, and the peak area of diosgenin was measured according to the above chromatographic conditions. Linear regression was performed with the reference concentration (X) as the abscissa and the peak area (Y) as the ordinate, and the regression equation  $Y=0.1366X+44.658$  ( $r=0.9993$ ). The results showed that diosgenin had a good linear relationship in the range of  $100 \sim 1500 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$ .

## Melting point detection of diosgenin

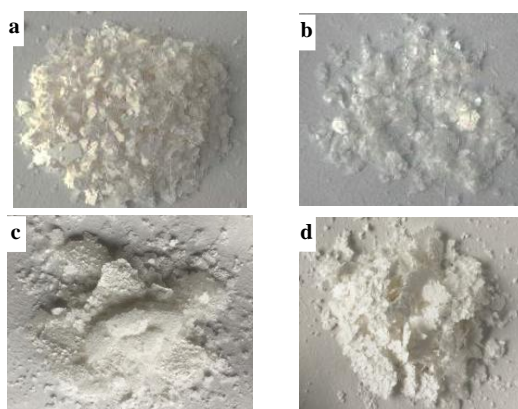
B-type melting point tube was used for melting point detection, and the specific detection method was referred to

**Table 1:** Results of composition comparison of four kinds of dioscorea rhizomes.

Composition	Diosgenin	Fiber	Starch	Watersoluble	Others
DNM/%	3.21	32.63	35.86	20.66	7.64
DC/%	4.50	42.29	25.47	18.90	8.84
WDZ/%	5.63	25.46	30.48	28.19	10.24
PDZ/%	3.28	24.91	34.18	30.28	6.73

**Table 2:** Results of recovery rate of fiber, starch and the yield of diosgenin from four kinds of dioscorea rhizomes.

Component	DNM	DC	WDZ	PDZ
Recovery rate of fiber/%	56.25	59.38	58.06	61.22
Recovery rate of starch/%	66.93	66.12	55.23	46.17
Yield rate of diosgenin /%	94.64	95.28	97.75	94.43



**Figure 3:** Four kinds of diosgenin products (a DNM, b DC, c WDZ, d PDZ).

"melting point determination method" in the appendix of pharmacopoeia of the People's Republic of China (National Pharmacopoeia Commission, 2015).

## RESULTS AND DISCUSSION

### Compositions of four dioscorea rhizomes

The compositions of the four dioscorea rhizomes were preliminarily tested (Peng, 2015), and the testing results are shown in Table 1. There are differences in contents of diosgenin in different dioscorea rhizomes. Even for the same kind dioscorea rhizomes, there are differences in contents of diosgenin in different growth times (Cao et al, 2004). The diosgenin content of wild *D. zingiberensis* is higher than others, and the water-soluble content of *D. zingiberensis* is significantly higher than that of *D. nipponica* Makino and *D. composita*.

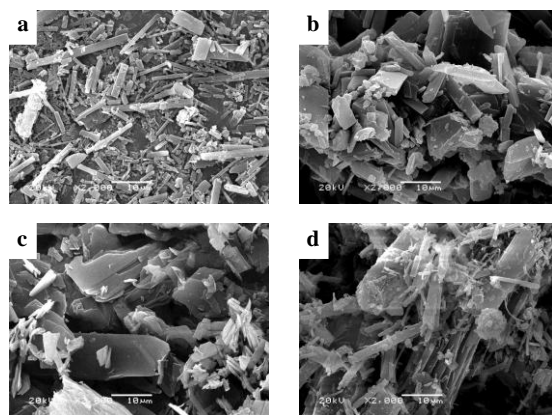
### Yield of fiber, starch and diosgenin

The recovery rate of dioscorea fiber, starch and the yield of diosgenin are shown in Table 2. About 50% of the fiber and starch has been recycled, and can be used for resource reuse. The yield of diosgenin from wild *Dioscorea zingiberensis* was higher than that from others.

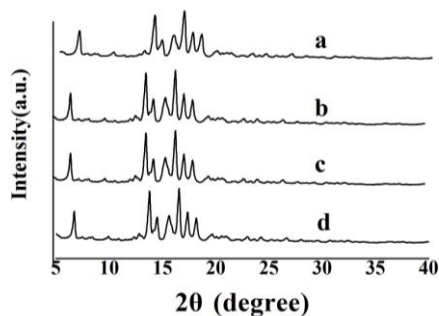
### Physical and chemical analysis of diosgenin products

#### Diosgenin products

Diosgenin products prepared from the four dioscorea rhizomes are shown in Figure 3. As shown in the figure, all are white and consistent with industry standards. There is a



**Figure 4:** Micromorphologies of four kinds of diosgenin products under SEM (a DNM, b DC, c WDZ, d PDZ).



**Figure 5:** XRD of four kinds of diosgenin (a DNM, b DC, c WDZ, d PDZ).

certain amount of yellow pigment in *Dioscorea* rhizomes, especially *D. zingiberensis*, which has a great influence on the color of diosgenin products. The diosgenin products prepared by the existing preparation processes are all yellowish to varying degrees, so a process to remove yellow pigment is very necessary to be added. At present, there is no better process to remove yellow pigment except using organic solvent dissolution or resin adsorption, which would cause loss of diosgenin and take away the profit from enterprises (Li et al., 2010); therefore, it becomes a major problem faced by *D. zingiberensis* diosgenin manufacturers. Through the independently developed water separation process in our laboratory, no additional process steps are required to remove the yellow pigment, and the *D. zingiberensis* diosgenin products are white and consistent with industry standards. This innovative process is suitable for the preparation of various *Dioscorea* rhizomes as material.

### **Micromorphologies of diosgenin products**

The micromorphologies of the four kinds of diosgenin

products under SEM are shown in **Figure 4**. Under the magnification of 2000 times, the four diosgenin products were all blocky, which were products of rapid crystallization.

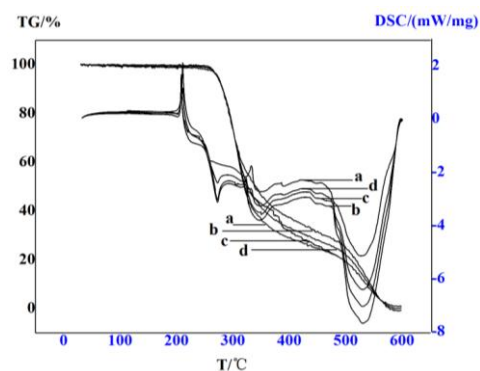
### **XRD analysis of diosgenin products**

The XRD detection and analysis diagram of the four kinds of diosgenin products are shown in **Figure 5**. The figure shows crystal characteristics, and the peaks of the four kinds of diosgenin products almost overlap.

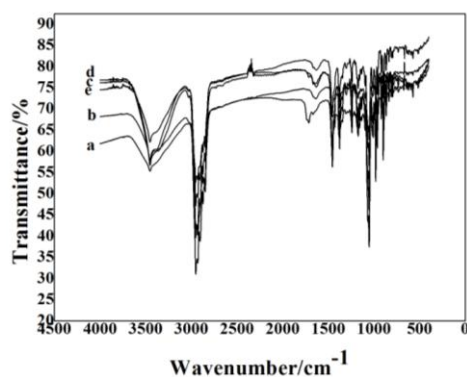
### **TG-DSC analysis of diosgenin products**

The TG-DSC curves of four kinds of diosgenin products are shown in **Figure 6**. One obvious endothermic peak appears on the DSC curves of the four diosgenin products, which is judged as the melting point peak because it is not accompanied by weight loss basically. Three obvious endothermic peaks appear on the DSC curve. First one is a weaker endothermic peak near 270°C which is generated by the pyran ring bond broken. Second endothermic peak near





**Figure 6:** TG-DSC of four kinds of diosgenin (a DNM, b DC, c WDZ, d PDZ).



**Figure 7:** Infrared spectrum of four kinds of diosgenin and standard (a standard, b DNM, c DC, d WDZ, e PDZ).

342° is caused by other ring bonds opening gradually. The last endothermic peak near 520°C is a strong peak formed by the volatile products of small and medium molecules during depolymerization. The TG curves of four kinds of diosgenin products have a weight loss of 2% before 265°C, which is mainly caused by water volatilization. After 265°C, the rapid weight loss begins in the temperature range of 265~335°C, and the weight loss of samples reaches 60%. This stage is the rapid decomposition of diosgenin. Due to sufficient oxygen supply in the air atmosphere, the rapid pyrolysis of diosgenin generates a large amount of CO<sub>2</sub>, so the decomposition is slow down in the temperature range of 335~512°C, and the weight loss of samples in this stage is 24%. After 512°C, the diosgenin samples are carbonized and produce CO, and the weight loss rate are accelerated to almost 100%.

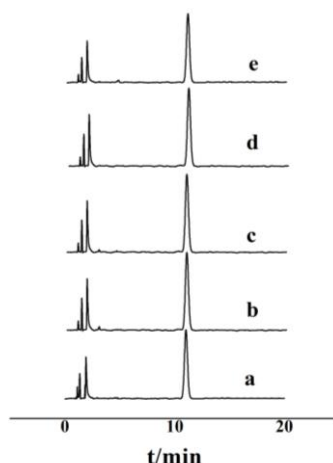
#### **FT-IR analysis of diosgenin products**

The FT-IR analysis was used to characterize four kinds of

diosgenin samples as shown in **Figure 7**. The characteristic absorption peak of four kinds of diosgenin products almost overlap, which have 3400 cm<sup>-1</sup>(Stretching vibration absorption peak of -OH), 2950 cm<sup>-1</sup> (Stretching vibration absorption peak of -CH), 1650 cm<sup>-1</sup> (Deformation vibration absorption peak of -OH), 1450 cm<sup>-1</sup>(Bending vibration peak of C-H), 1150 cm<sup>-1</sup>(Stretching vibration absorption peak of -CH<sub>3</sub>), 1050 cm<sup>-1</sup>(Stretching vibration absorption peak of ether bond (C-O) in the five-element ring), and 980 cm<sup>-1</sup>(Stretching vibration absorption peak of ether bond (C=C) in pyran ring). Thus, all basically overlap with diosgenin standard spectrogram.

#### **Purity of diosgenin products**

HPLC spectra of four kinds of diosgenin products and standard products as shown in **Figure 8**. The purity of the four diosgenin products was 95.25, 95.37, 96.09 and 95.19% respectively, which all met the industry standard (≥95%). Wild *D. zingiberensis* has high content of diosgenin



**Figure 8:** HPLC spectra of four kinds of diosgenin products and standard (a standard, b DNM, c DC, d WDZ, e PDZ).

**Table 3:** Melting points of four kinds of diosgenin products.

Item	DNM	DC	WDZ	PDZ
melting range /°C	195~205	195.5~204.5	197~203	195.5~205

and good quality. However, its resources are basically exhausted, and now enterprises preparing diosgenin usually use cultivated *D. zingiberensis* as raw materials. Because the growing year of cultivated *D. zingiberensis* is basically 2~3 years, the content of diosgenin in the rhizomes was significantly lower than that of wild *D. zingiberensis*, but the purity of diosgenin prepared by water separation is also in line with the industry standard.

### **Melting points of diosgenin products**

Melting points of four diosgenin products were detected, and the results are shown in Table 3. The melting points of diosgenin products were all higher than 195°C, and this in line with the industry standard ( $\geq 194^\circ\text{C}$ ). The melting point of wild *D. zingiberensis* is relatively high, and the purity is the same.

### **CONCLUSIONS**

(1). Four kinds of diosgenin products were obtained by water separation saponin and high temperature and pressure hydrolysis with the extraction rate of 94%.

(2). The four kinds of diosgenin products are all white solid, which are all blocky by SEM micromorphology observation, and they are rapid crystallization products. By XRD, TG-DSC,

FT-IR detection and analysis, their peaks almost overlap, and consistent with the standard.

(3). Through HPLC and melting point detection, the purity of four diosgenin products is 95% or above, and the melting point is 195°C or above, they all meet the requirements of diosgenin industry.

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