Optimization of Enzyme-assisted Ultrasonic Extraction of Total Ginsenosides from Ginseng Roots

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Abstract. The ginseng root was used as the experimental material to study the extraction process of ginseng root saponins by enzyme-assisted ultrasonic method, and the optimum process conditions were obtained. The ginsenoside Re was used as the standard. The total ginseng root saponins were extracted by enzyme-assisted ultrasonic method. The optimal extraction process was determined by orthogonal test with the percentage of total ginseng root saponins as indicators. The optimal combination of enzyme-assisted ultrasonic extraction of total ginsenosides was determined to be $A_2B_2C_3$: pH6.0, enzymatic hydrolysis temperature 35℃, and solid-liquid ratio 1:20. The extraction rate of total ginsenosides from ginseng roots can be improved by enzyme-assisted ultrasonic method.

Introduction

Panax ginseng C.A. Mey. is a herb of Panax ginseng, and most of its active ingredients are concentrated in the main roots, leaves and flower buds [1]. Shengjuan SHAO et al [2] extracted the total flavonoids in the pollen of Pinus massoniana by ultrasonic coenzyme method, and found that the extraction rate of this method was improved compared with the traditional extraction process. Jinying WEI [3] effectively improved the extraction rate of various substances by using cellulase for the extraction process of paeoniflorin, alkaloid and paeonol in white peony, fritillary and peony bark. In this paper, ginseng roots were used as experimental materials to extract ginseng root saponins by enzyme-assisted ultrasonic method. The optimal extraction process was determined by orthogonal test, in order to improve the extraction rate of total ginsenosides by ginseng-assisted ultrasonic method.

Material

5-year-old ginseng (Zuojia Campus Medicine Plantation Park).

Ginsenoside Re (Shanghai Chunyou Biotechnology Co., Ltd.), cellulase R-10, methanol, 5% vanillin-glacial acetic acid solution, 8% vanillin ethanol solution, perchloric acid, glacial acetic acid, 77% sulfuric acid , absolute ethanol, water saturated n-butanol, ether and so on.

Multi-function pulverizer; THC type CNC ultrasonic extractor; YP5001 electronic balance; 752 UV-visible spectrophotometer; C20 glass instrument dryer; circulating water multi-purpose vacuum pump SHB-IIIA.

Method

Extraction process of total ginsenosides from ginseng root: ginseng root→drying (45℃)→grinding (over 40 mesh sieve)→ginseng root powder→adding pH buffer solution and cellulase (enzyme activation)→enzymatic hydrolysis→ultrasonic extraction→boiling water (5min)→filter→filtrate evaporation at atmospheric pressure→ginseng root residue.

The ginseng root residue was reconstituted with 30 mL of water, transferred to a separatory funnel, and degreased twice with 15 mL of diethyl ether. The aqueous layer was extracted with water and saturated with n-butanol. The n-butanol solution was collected, and the solvent was evaporated under normal pressure to obtain 0.1 g of residue. The content of the methanol was

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measured after the volume was adjusted to 10 mL.

Drawing of ginsenoside Re standard curve: Accurately weigh ginsenoside Re 1.0mg, dilute to 10mL volumetric flask with methanol, shake and set aside.

Drawing of standard curves: Pipette the reference solution 0.00mL, 0.05mL, 0.10mL, 0.15mL, 0.20mL, 0.25mL, 0.30mL, 0.35mL into the test tube, drain the solvent in a water bath, and then add the newly prepared 5% vanillin-glacial acetic acid 0.2 mL of the solution and 0.8 mL of perchloric acid were shaken, and heated in a constant temperature water bath at 60℃ for 15 min, and then immediately cooled with running water for 2 min. Add 5 mL of glacial acetic acid and shake well. The full wavelength scan is performed at a wavelength of 400-800 nm using an ultraviolet spectrophotometer, and the maximum absorption wavelength is selected as the detection wavelength. Taking the absorbance as the ordinate, the concentration of ginsenoside Re (mg/ml) is plotted on the abscissa.

Determination of total saponin content in ginseng root: Take 0.3ml of the solution to be tested in a 10mL graduated test tube, add 0.2mL of methanol, take a 10mL graduated test tube, add 0.5mL of methanol, add 8% vanillin ethanol solution 0.5mL, 77% sulfuric acid 5mL in the ice water bath. Shake well, heat in a 60℃ water bath for 10 min, cool for 15 min, measure the absorbance A at the maximum absorption wavelength, and calculate the content. The total saponin concentration C (mg/ml) of ginseng root was obtained from the standard curve, and the total saponin content of ginseng root Y (mg/g) was calculated according to the formula [4]. The content of total ginsenosides of ginseng root was calculated according to the formula Y = [(C×30×10)/M]×100%.

Single factor test: Buffer solution with pH of 5.0, 5.5, 6.0, 6.5, 7.0; Enzymatic hydrolysis temperature 30, 35, 40, 45, 50℃; Material to liquid ratio is 1:10, 1:15, 1:20, 1:25, 1:30; The amount of enzyme used was 0.2, 0.8, 1.4, 2.0, 2.6%; Enzymatic hydrolysis time 30, 60, 90, 120, 150min; Ultrasonic time 5, 10, 15, 20, 25min.

Orthogonal test of enzyme-assisted ultrasonic extraction of total ginsenosides from ginseng root: On the basis of single factor experiment, the pH value (A), enzymatic hydrolysis temperature (B) and solid-liquid ratio (C) were used as orthogonal factors to extract the percentage of total ginseng root saponins as indicators, and each factor took 3 Horizontal, select L_9 (3^4) orthogonal table for orthogonal test, orthogonal test design table, see Table 1.

Table1 Orthogonal design table of ginseng root saponins test

Horizontal factor	A(pH)	B(℃)	C(g/ml)
1	5.5	30	1:10
2	6.0	35	1:15
3	6.5	40	1:20

Optimization process verification: The ginseng root saponins were extracted by the best process and repeated experiments were carried out 3 times [5], and the test results were verified.

Test Results

Standard curve of ginsenoside Re: From the measured results, the maximum absorption wavelength is 554 nm, and the standard curve is determined at this wavelength. The regression equation between the concentration of ginsenoside Re and the absorbance value shown in Fig.1 is $y=21.9x+0.0181$, $R^2=0.9997$.

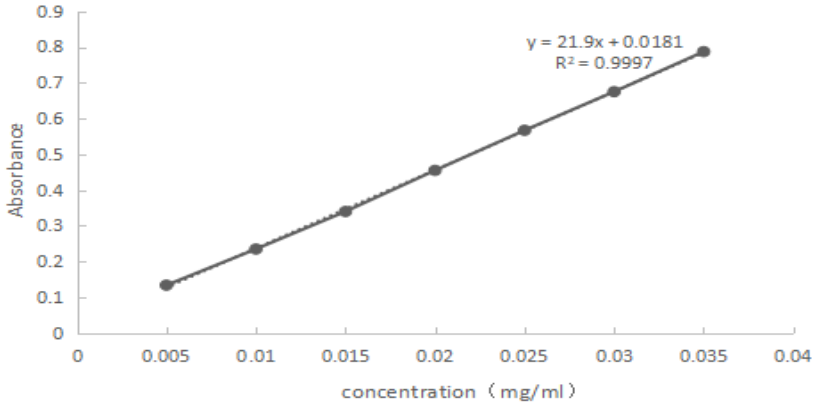

Fig.1 Standard curve of ginsenoside Re

Effect of pH on the percentage of total saponins extracted from ginseng root:

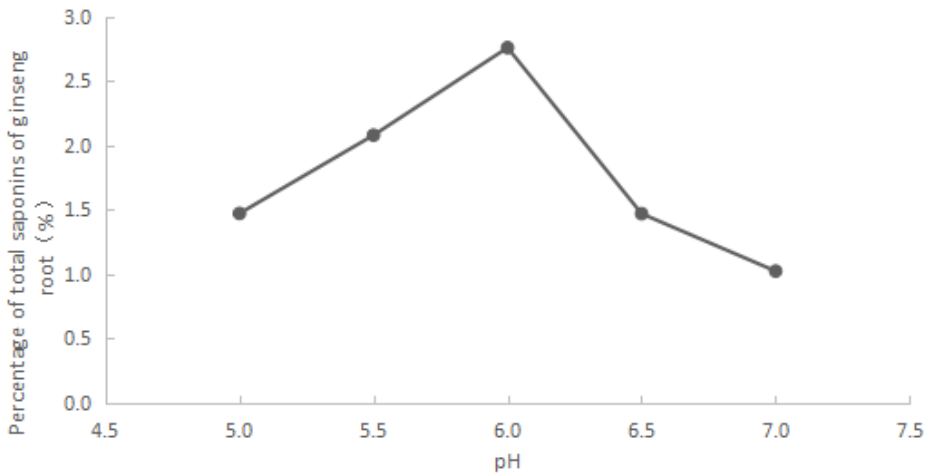

Fig.2 Effect of pH on the percentage of total saponins extracted from ginseng root

As shown in Fig.2, when the pH value is 6.0, the total ginsenoside percentage value of the ginseng root reaches the maximum value, because the cellulase activity is the largest at pH 6.0, which plays a high-efficiency catalytic role. After analysis of variance, $F=40.191>F_{0.01}(4,10)$, indicating that there is a significant difference in the percentage of total ginsenosides extracted from ginseng roots at $F_{0.01}$. This factor will be used as an investigation factor in orthogonal test.

The effect of enzymatic hydrolysis temperature on the percentage of total ginsenosides extracted:

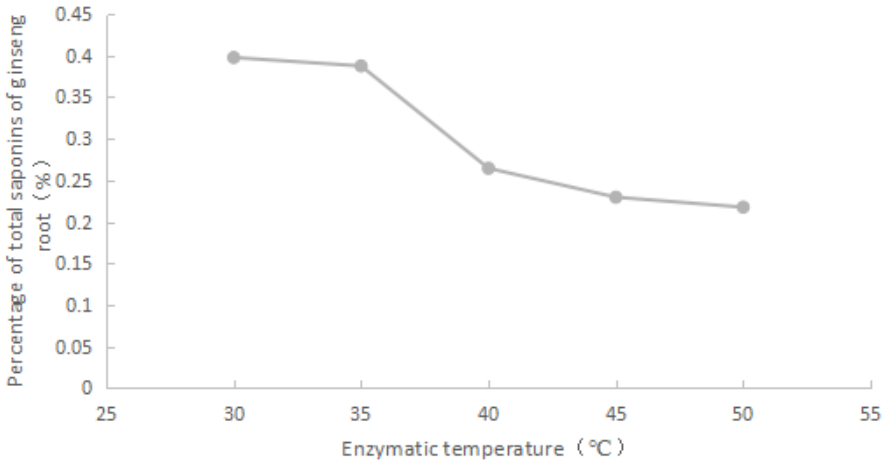

Fig. 3 Effect of enzymatic hydrolysis temperature on the percentage of total ginsenosides extracted from ginseng

As shown in Fig.3, when the enzymatic hydrolysis temperature reaches 30℃, the percentage of total ginsenosides of ginseng root reaches the maximum value. This is because the cellulase activity

is high and the enzymatic hydrolysis rate is fast, which the dissolution rate of total ginsenosides of ginseng root is improved. After variance analysis, $F=585.901>F_{0.01}(4,10)$, indicating that the enzymatic hydrolysis temperature has significant difference in the percentage of total ginseng root saponins extracted at $F_{0.01}$. This factor will be used as an orthogonal test factor.

Effect of liquid-liquid ratio on the percentage of total ginsenosides extracted from ginseng root:

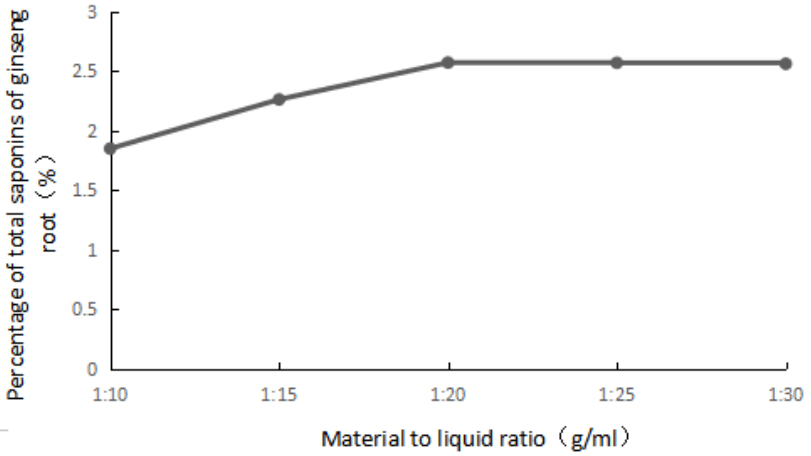

Fig. 4 Effect of ratio of material to liquid on the percentage of total ginsenosides extracted from ginseng

As shown in Fig.4, the percentage of total ginsenosides in ginseng roots increases with the increase of solid-liquid ratio. When the ratio of material to liquid is 1:15, the percentage of total ginsenosides extracted is the largest, because the amount of solvent is larger. The difference in concentration of the active ingredient from the ginseng tissue into the solvent is greater, which is beneficial to the diffusion and mass transfer of the active ingredient. However, after the amount of solvent reaches a certain level, the percentage of total ginsenosides extracted from ginseng roots tends to be stable. After variance analysis, $F=1003.107>F_{0.01}(4,10)$, indicating that there is a significant difference in the percentage of total ginsenosides extracted from ginseng roots at $F_{0.01}$. This factor will be used as an orthogonal test factor.

Effect of enzyme dosage on the percentage of total ginsenosides extracted from ginseng root: After analysis of variance, $F=3.149<F_{0.05}(4,10)$, indicating that there is no significant difference in the amount of total saponins extracted from ginseng roots at $F_{0.05}$.This factor will not be used as an investigation factor for orthogonal experiments.

Effect of enzymatic hydrolysis time on the percentage of total saponins extracted from ginseng root: After variance analysis, $F=2.769<F_{0.05}(4,10)$, indicating that the enzymatic hydrolysis time has no significant difference in the percentage of total ginsenosides extracted at $F_{0.05}$. This factor will not be used as an investigation factor for orthogonal experiments.

Effect of ultrasonic time on the percentage of total saponins extracted from ginseng root: After variance analysis, $F=0.985<F_{0.05}(4,10)$, indicating that there is no significant difference in the percentage of total saponins extracted from ginseng roots at $F_{0.05}$. This factor will not be used as an investigation factor for orthogonal experiments.

Orthogonal test results of total saponins of ginseng root:

Table 2 Orthogonal test results of total ginsenoside percentage of ginseng root

Test number	A	B	C	Test plan	Percentage of total saponins of ginseng root (%)
1	1	1	1	$A_1B_1C_1$	0.1092
2	1	2	2	$A_1B_2C_2$	2.0868
3	1	3	3	$A_1B_3C_3$	0.3336
4	2	1	2	$A_2B_1C_2$	0.0384
5	2	2	3	$A_2B_2C_3$	2.0436
6	2	3	1	$A_2B_3C_1$	1.1400
7	3	1	3	$A_3B_1C_3$	1.5504
8	3	2	1	$A_3B_2C_1$	0.0984
9	3	3	2	$A_3B_3C_2$	1.4460

K_1	2.5296	1.6980	1.3476
K_2	3.2220	4.2288	3.5712
K_3	3.0948	2.9196	3.9276
K_1	0.8432	0.5660	0.4492
K_2	1.0740	1.4096	1.1904
K_3	1.0316	0.9732	1.3092
R	0.2308	0.8436	0.7412

It can be seen from Table 2 that the influence degree of each factor on the extraction of total ginsenosides is B>C>A, and it is concluded that the enzymatic hydrolysis temperature is the main influencing factor, and the effect of enzymatic hydrolysis pH is the least. The optimum combination was determined to be A2B2C3, that is, the pH was 6.0, the hydrolysis temperature was 35℃, and the ratio of material to liquid was 1:20.

Analysis of variance of ginseng root saponins in orthogonal test: F_C=19.3315>$F_{0.05,}$ which shows that the ratio of material to liquid has a significant effect on the extraction rate of total ginsenosides.

The best process verification result of total ginsenosides of ginseng root: The results from the parallel experiments were essentially parallel. The relative standard deviation RSD=1.036, which indicates that the optimal process conditions selected by the orthogonal test have good repeatability.

Conclusion

The total extraction percentage of ginseng root saponins was 2.939% after multiple extractions. Compared with the total extraction of ginseng root saponins by enzymatic extraction, the extraction method of ginseng total saponins was higher. This experiment optimized the process of extracting total ginsenosides from ginseng roots and achieved the expected results. The cellulose tissue in ginseng cells is mainly destroyed by cellulase, so that the total saponins of ginseng are dissolved from the cells, so that the extraction rate is improved, and the extraction process is simple and easy, and the requirements on technology and equipment are not high.

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