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Ginsenoside extraction from *Panax quinquefolium* L. (American ginseng) root by using ultrahigh pressure

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Abstract

A new method of ultrahigh pressure extraction (UPE) was used to extract the ginsenosides from *Panax quinquefolium* L. (American ginseng) root at room temperature. Several solvents, including water, ethanol, methanol, and *n*-butanol were used in the UPE. The ginsenosides were quantified by a HPLC equipped with UV–vis detector. The results showed that ethanol is the most efficient solvent among the used ones. Compared with other methods, i.e., Soxhlet extraction, heat reflux extraction, ultrasound-assisted extraction, microwave-assisted extraction, and supercritical CO_2 extraction, the UPE has the highest extraction yield in the shortest time. The extraction yield of 0.861% ginsenoside-Rc in 2 min was achieved by the UPE, while the yields of 0.284% and 0.661% were obtained in several hours by supercritical CO_2 extraction and the heat reflux extraction, respectively.
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Keywords: High hydrostatic pressure; Extraction; Natural products; Ginsenoside; *Panax quinquefolium* L

1. Introduction

Extraction is the first essential step for the isolation and purification of many bioactive components from the natural products. To determine the biomaterial components, the components must be firstly transferred into solution, i.e. extraction is the first step for biomaterial analysis. There are many extraction methods, such as Soxhlet, heat reflux, ultrasound-assisted, microwave-assisted, and supercritical CO_2 extraction, and so on.

Ultrahigh pressure extraction (UPE), also called cold ultrahigh isostatic hydrostatic pressure extraction, is a new extraction technique. The ultrahigh pressure technology has been widely used in ceramics, graphite, casting industry, pharmaceutics, metallurgy, plastic making, civil engineering, and food industry. In recent years, the ultrahigh pressure has been used for extraction of Chinese medicine [1]. The operating process of UPE is similar to high pressure process of food, i.e., materials are firstly mixed with solvents at room temperature, with or without packaging, then the liquid mixture is pressured between 100 and 1000 MPa for a certain time and then the pressure is released quickly. Studies show that UPE has many advantages such as shorter processing time, higher extraction yield, lower power consumption, less impurity in the extraction liquid, operation at room temperature, particularly the qualities of extraction such as nutrient retention, activity and structure of the essential components are generally not adversely affected by the pressure process [2]. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents, heat and/or agitation to increase the solubility of the essential components and the rate of the equilibrium. Since UPE is carried out at room temperature, the thermal damage and loss of volatile components can be avoided. Heat reflux and supercritical CO_2 methods usually need several hours, while UPE only needs a few minutes. Compared with supercritical fluid extraction [3], the equipments of ultrahigh pressure extraction are simpler and cheaper, and various solvents can be used by UPE.

Ginsenosides, known as the principal bioactive components of *Panax quinquefolium* L. (American ginseng) have been widely used for health foods and traditional medicine [4]. Among ginsenosides, Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 are the main compounds (Fig. 1). Purified ginsenosides are very expensive due to their low yield in *P. quinquefolium* L. roots. It is very important to develop quick and efficient extraction and

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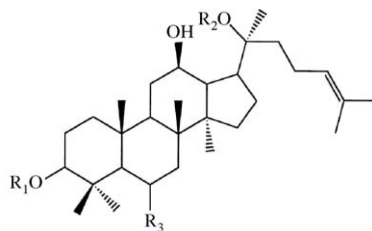

Ginsenosides	R_1	R_2	R_3	Formula	Molecular weight
Ginsenoside Rb_1	-Glc^2-Glc	-Glc^6-Glc	-H	$C_{54}H_{92}O_{23}$	1108
Ginsenoside Rb_2	-Glc^2-Glc	-Glc^6-Ara(p)	-H	$C_{53}H_{90}O_{22}$	1078
Ginsenoside Rc	-Glc^2-Glc	-Glc^6-Ara(f)	-H	$C_{53}H_{90}O_{22}$	1078
Ginsenoside Rd	-Glc^2-Glc	-Glc	-H	$C_{48}H_{82}O_{18}$	946
Ginsenoside Re	-H	-Glc	-O-Glc^2-Rha	$C_{48}H_{82}O_{18}$	946
Ginsenoside Rg_1	-H	-Glc	-O-Glc	$C_{42}H_{72}O_{14}$	800

Glc, glucose; Ara(p), arabinose in pyranose form; Ara(f), arabinose in furanose form; Rha, rhamnose

Fig. 1. Chemical structures of ginsenosides.

purification technique to reduce cost and enhance their medical applications.

In this work, the ultrahigh pressure technology was used for ginsenosides extraction from *P. quinquefolium* L. (American ginseng) root. The aim of this study is to find an efficient method of ginsenosides extraction from *P. quinquefolium* L. The effects of pressure, solvent concentration, ratio of solvent to material, and extraction time on the extraction efficiency were investigated. The results might provide useful information for the production of high quality ginsenoside extracts.

2. Experimental

2.1. Materials and reagents

Ginsenoside Rb_1, Rb_2, Rc, Rd, Re, and Rg_1, as the standard samples, were obtained from Foundation Medical College of Jilin University. A 4-year-old *P. quinquefolium* L. roots were cultivated in Jilin Province of China. The acetonitrile and methanol were HPLC grade (Fisher Scientific, USA). Analytical grade ethanol, *n*-butanol and trichloromethane were obtained from Beijing Chemical Reagent Factory. Water was purified by Milli-Q system (Millipore, Bedford, MA, USA). All solutions were filtered through a 0.45 μm hydrophilic polypropylene membrane before use.

2.2. Apparatus

DL700 ultrahigh pressure machine (Da Long Machine Factory, Shanghai, China) was used for the UPE processing. The used high performance liquid chromatography (HPLC) system (Shimadzu, Japan) comprises the following components: SCL-10A vp system controller, four LC-10AT vp pumps, SIL-10ADvp automatic sample injector with sample cooler, CTO-10ASvp column oven, DGU-14A degasser, and SPD-M10A VP ultraviolet–visible (UV–vis) photodiode array detector. Shimadzu Class-VP Chromatography Laboratory Automated Software System, Version 5.03 was used for system operation, data collection and reprocessing.

2.3. Sample preparation

The roots of *P. quinquefolium* L. were dried in vacuum at 60 °C for 24 h, then pulverized and sieved with 40 mesh screen. Powders of *P. quinquefolium* L. root with particle size under 40 mesh were obtained. In order to remove the fat contents, the powders were mixed with trichloromethane, the ratio of solvent (ml) to material (g) was 40–1. The liquid mixture was refluxed for 3 h, and then the trichloromethane was distilled. The residues were dried at room temperature and used as the test samples.

2.4. Quantitative analysis method

The mixture of standard samples (ginsenoside) or the sample of extraction was dissolved in 10 ml of methanol and filtered through filters [0.45 mm (Millipore)] for HPLC analysis. The analytical column was performed on a Diamonsil C18, 5 μm, 250 mm × 4.6 mm, at 35 °C. The separation of ginsenosides was obtained by gradient elution. Eluents was the mixture of A (acetonitrile) and B (0.05% of phosphoric acid aqueous solution). The process of eluent is according to the following profiles: 0–20 min, VF (volume fraction): 80–78%; 20–45 min, VF: 72–55%; 45–55 min, VF: 55–45%; 55–60 min, VF: 45–0%; 60–65 min, VF: 0–80%. The flow rate was kept at 1.0 ml/min. Injection volume was 20 μl. The absorbance was measured at wavelength of 203 nm for the detection of ginsenosides.

2.5. Ultrahigh pressure extraction

The sample of *P. quinquefolium* L. roots was mixed with the solvents, and the ratio of solvent (ml) to material (g) is in the range of 10–100. The mixture was poured into a plastic bag, and the bag was sealed by heating and subjected to ultrahigh pressure treatment at selected pressures in the range of 100–600 MPa

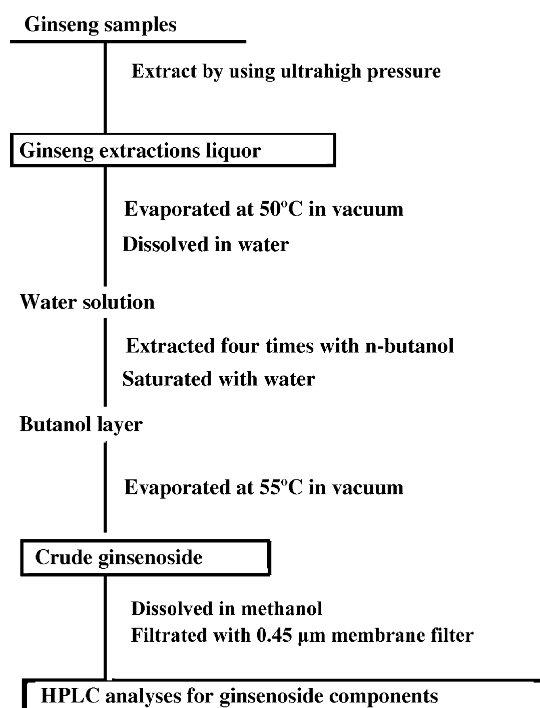

Fig. 2. Procedures for the extraction, separation, and determination of ginsenosides from ginseng samples.

for different durations (1–5 min). The pressure was then quickly released. The extractions were filtered through Whatman 40 filter. The filtrates were evaporated on a rotary evaporator at 50 °C. The evaporated residue was dissolved in 20 ml distilled water and was extracted for four times with 20 ml water-saturated *n*-butanol. The butanol solution was washed twice with 20 ml distilled water to remove the impurities. The remaining butanolic solution was transferred to a tarred round bottom flask to evaporate by a rotary evaporator under vacuum at 55 °C, and the residues were dissolved in 40 ml methanol. The amount of extracted ginsenoside was determined by HPLC and the yield (%) was calculated. The flow chart is shown as Fig. 2.

2.6. Other extraction methods

It should be mentioned that the operation conditions of each method described below was operated at the optimum conditions which were assessed by the highest yield determined through experimental design.

2.6.1. Ultrasound-assisted extraction

One-gram sample of *P. quinquefolium* L. root was put into a 100 ml conical flask. After adding 50 ml of 70% ethanol–water solution (v/v), the flask was sonicated for 40 min in an ultrasonic bath (frequency 50 Hz, power 250 W). The separation and determination of ginsenosides followed the procedure shown in Fig. 2.

2.6.2. Microwave-assisted extraction

One-gram sample of *P. quinquefolium* L. root was put into a 100 ml conical flask. After adding 50 ml of 70% ethanol–water solution (v/v), the flask was exposed to the microwave. The microwave extractor was operated at 300 W with an emission frequency of 2450 MHz under atmospheric pressure condition and the extraction was carried out for 15 min. The separation and determination of ginsenosides followed the procedure shown in Fig. 2.

2.6.3. Supercritical CO_2 extraction

HA221-50-06 Supercritical Fluid Equipment (Huaan Supercritical Fluid Extractor Limited Company, Jiangsu, China) was used and 3% (v/v) ethanol was mixed in the supercritical CO_2. The extraction was operated at 40 °C and 30 MPa for 4 h. The flow rate of solvent was 2 l/min. The amount of extracted ginsenosides was determined by HPLC and the yield (%) was calculated.

2.6.4. Soxhlet extraction

One-gram sample of *P. quinquefolium* L. root was put into a 50 ml Soxhlet thimble. The apparatus was fitted with a 100 ml round-bottom flask containing 50 ml of 95% ethanol–water solution and a boiling regulator. The flask was heated at 70 °C in a water bath and the solvent was refluxed 8 h. The separation and determination of ginsenosides followed the procedure shown in Fig. 2.

2.6.5. Heat reflux extraction

One gram sample of *P. quinquefolium* L. root mixed with 50 ml of 50% ethanol–water solution in a 150 ml round bottom flask fitted with a cooling condenser which was used to perform the extraction. The extraction temperature was controlled at 70 °C with a water bath to allow ethanol boiled continuously. Extraction was carried out for 6 h. The separation and determination of ginsenosides followed the procedure shown in Fig. 2.

3. Results and discussion

3.1. Identification of ginsenosides

HPLC was used to analyze the ginsenoside. The HPLC peaks were identified by comparing their retention times with those of the samples of standard and extraction, which was determined by same chromatographic conditions. The chromatograms were shown in Fig. 3.

The linear calibration curves, and concentration range of ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 were shown in Table 1, where y is the amount of ginsenoside and x is the area of perk.

3.2. The effect of solvent

Several solvents were used to extract ginsenoside from *P. quinquefolium* L. root, such as water, ethanol–water solution, methanol–water solution, water-saturated *n*-butanol. Due to the different of polarities of these four extraction solvents, the solubility of ginsenosides and the rate of mass transfer are different. Fig. 4 showed the yields of ginsenosides extracted by UPE with different extraction solvent. It can be seen that the extraction

Table 1
Linear calibration curve and concentration range of different ginsenosides

Ginsenosides	Calibration curve, y	Concentration range (mg/ml)	Correlation coefficient, r^2
Rg_1	$1.86846E\text{-}07x - 4.02232E\text{-}02$	0.023–0.310	0.999
Re	$2.37396E\text{-}07x - 4.51656E\text{-}03$	0.023–0.310	0.997
Rb_1	$3.48217E\text{-}07x - 8.71567E\text{-}03$	0.023–0.310	0.998
Rc	$2.25100E\text{-}07x - 7.67859E\text{-}03$	0.027–0.354	0.997
Rb_2	$1.89891E\text{-}07x - 2.23323E\text{-}02$	0.035–0.388	0.999
Rd	$2.01361E\text{-}07x - 2.47141E\text{-}02$	0.047–0.630	0.999

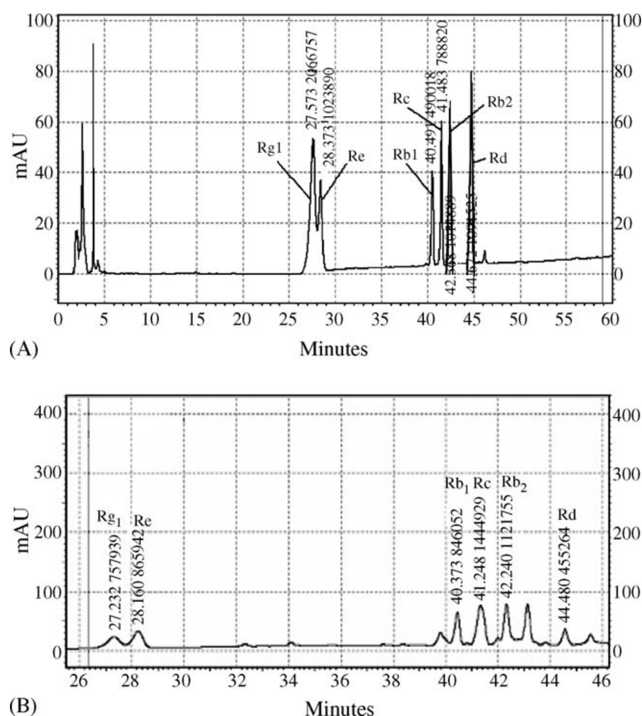

Fig. 3. HPLC figure of the ginsenosides (A: solution of standard samples and B: extraction of *P. quinquefolium* L. root).

yields of ginsenosides in ethanol–water solution are the highest and the extraction yields in water is the lowest. Ethanol is non-toxic, and it is easy to be recycled and mix with water in different ratios. As an extracting solvent, the *n*-butanol has a higher boiling point (117.8 °C) than ethanol, which makes it more difficult to evaporate. The higher evaporating temperature may cause thermal decomposition of some ginsenosides, especially the malonyl ginsenosides which are thermally unstable at higher temperature [5]. The optimal extraction solvent is ethanol–water for UPE.

3.3. The effect of ethanol concentration

To investigate the effect of ethanol concentration, UPE were performed at 200 MPa for 2 min with ethanol–water solution of various concentration, the ratio of solvent (ml) to the sample (g) was 50.

Fig. 5 showed the effect of ethonal concentration on the extraction yield of ginsenoside from *P. quinquefolium* L. root. The extraction yield of ginsenoside improved with the increasing of the ethanol concentration in the range of 10–70%. When the ethanol concentration is higher than 70%, the extraction yield of ginsenoside decreased slowly with increasing of ethanol concentration. It is known that the solubility of neutral and malonyl ginsenosides are varied in different concentration ethanol. Therefore, the breakage degree of the cell membrane is different in different concentration ethanol. The protein could be coagulated in higher concentration ethanol, making larger diffusion resistance. It was reported [6] that the maximum extraction of neutral, malonyl, and total ginsenosides was obtained with 70%, 40%, and 60% ethanol, respectively.

3.4. The effect of pressure

Pressure is one of the most important parameters in UPE, and it is directly correlated to the solubility of ginsenoside. A higher extracting pressure is profitable for the good yield or reduction of extraction time. It was found that the yield increased linearly with pressure in the range of 100–500 MPa. When the pressure was higher than 500 MPa, the yield decreased slightly.

Considering the higher cost and lower safety of the higher pressure equipment for UPE and the small different of yields,

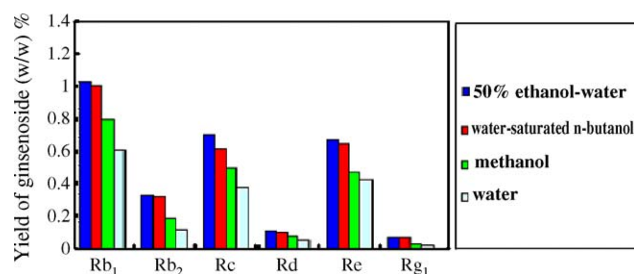

Fig. 4. The effect of different solvents in UPE (experimental conditions: pressure – 200 MPa, ratio of solvent (ml) to the sample (g) – 50, and time – 2 min).

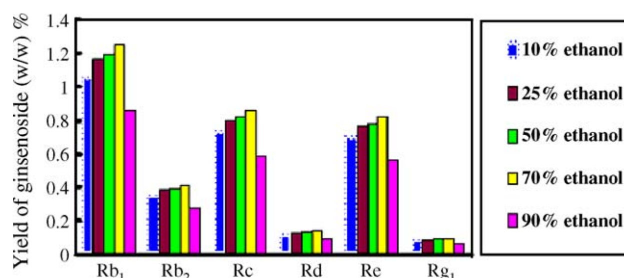

Fig. 5. The effect of ethanol concentration on UPE.

the pressure of 200 MPa was chosen for ginsenosides extraction in this work.

3.5. The effect of ratio of solvent to material

The sample of *P. quinquefolium* L. root was extracted at 200 MPa for 2 min in 50% ethanol–water solution. The results showed that the yield increased linearly with the ratio of solvent (ml) to material (g) in the range of 10–75.

It can be imagined that there are more chances for the solid phase to contact with liquid phase in UPE at high solvent/material ratio. However, the higher the ratio of solvent to material means that more solvent would be consumed, which makes more difficult to evaporate the solvent. A 50:1 of the solvent/material (ml/g) ratio was chosen to extract ginsenosides from *P. quinquefolium* L. root by UPE.

3.6. The effect of extraction time

The sample of *P. quinquefolium* L. root was extracted with 70% ethanol–water solution at 200 MPa.The holding time of UPE were 1, 2, 3, 4, and 5 min, respectively. The results showed that the yield of ginsenosides has no obvious increasing with the extraction time (1–5 min). Under high pressure, the diffusion speed is very high. A 2 min is enough to extract ginsenosides from *P. quinquefolium* L. root by UPE.

3.7. Optimization of ultrahigh pressure extraction using experimental design

The experimental design method was used for more efficient optimization of the various operation variables in the UPE. Based on the above results, three main factors were chosen in the UPE of Ginsenoides, i.e., extraction pressure, ethanol concentration, and ratio of solvent to material (ml/g).

Uniform Design's Software V4.0 was used to plan the test and analyze the results. The test project and results are shown in Tables 2 and 3.

The optimum regression equation is shown below, which is also provided by the Uniform Design's Software V4.0:

$$Y = 0.3417486 + 0.0008669P + 0.0066393C + 0.0038203R - 0.0000005P^2 + 0.0000001PC - 0.0000091PR - 0.000093C^2 + 0.0000242CR$$

Table 2
Project of U_{10} (10^3) uniform design and test result CD = 0.0148

Number	Pressure (MPa)	Concentration (%)	Ratio (ml/g)	Yield of ginsenoside-Re (%)
1	300	90	10	0.436
2	500	10	10	0.701
3	600	30	75	0.727
4	500	90	100	0.641
5	400	50	50	0.772
6	300	10	100	0.746
7	600	70	25	0.694
8	400	50	50	0.784
9	200	30	25	0.679
10	200	70	75	0.782

Table 3
The results of variance analysis

Variance source	Square sum	Freedom degree	Even square	F	P
SR	0.0952	8	0.0119	165.3604	0.060076
SE	0.0001	1	0.0001		
ST	0.0953	9			

F: test method; *P*: significantly difference; ST: error.

with $r^2 = 0.9992$, $F = 165.3604 > F_{0.1}(8,1) = 59.4$, significant at $P < 0.10$

In which, P is the pressure (MPa), C the concentration of ethanol (%), and R is the ratio of solvent to sample (ml/g).

The optimum extraction conditions to extract Ginsenoide-Re by UPE are listed as follows: (I) ethanol concentration is 48.78%, (II) ratio of solvent to material (ml/g) is 100, and (III) pressure is 200 MPa; the forecast range of yield of ginsenoside-Re (%) by UPE is 0.3828–0.9159%.

According to the "function of model analysis" provided by the Uniform Design's Software V4.0, the relationship between the yield of ginsenoside-Re (%)(y) and the affecting factors are plotted as Fig. 6.

3.8. Comparison of UPE with other extraction methods

In order to compare with UPE, other extraction methods were also used. During the experiments, the amount of *P. quinquefolium* L. root sample and HPLC analysis were kept as the same as that in UPE. Each method was operated at the optimum

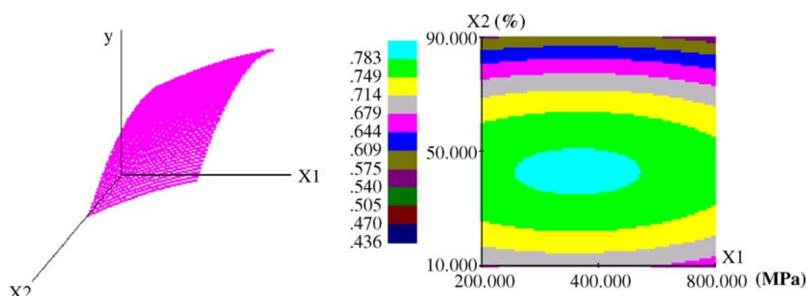

Fig. 6. The plot of Y depending on pressure and the concentration of solvent. $X1$ – pressure, $X2$ – concentration of solvent.

Table 4
Comparison of UPE with other extraction methods

Extraction methods	Solvent	Extraction time	Temperature (°C)	$\bar{y}$ ($n=3$)
Soxhlet	95% Ethanol	8 h	70	0.697
Heat reflux	50% Ethanol	6 h	70	0.761
Ultrasonic-assisted	70% Ethanol	40 min	50	0.716
Microwave-assisted	70% Ethanol	15 min	70	0.785
Supercritical CO_2	CO_2 + 3% ethanol (v/v)	4 h	40	0.324
Ultrahigh pressure	50% Ethanol	2 min	25	0.821

$\bar{y}$: yield of ginsenoside-Rc(%); n: times of repeating.

conditions which have the highest yield determined by running experimental design.

The results are shown in Table 4.

From Table 4 it can be seen that the extraction yield of ginsenoside-Rc by UPE for 2 min is much higher than that of Soxhlet extraction for 8 h, heat reflux extraction for 6 h, ultrasonic extraction for 40 min, microwave-assisted extraction for 15 min, and supercritical CO_2 extraction for 4 h. Therefore, UPE is the most efficient extraction methods compared with the others.

From Table 4 it also can be seen that among the six extraction methods, UPE can be carried out not only in the shortest time but also in the lowest temperature, therefore components of extraction by UPE will be a higher activity and purity.

The traditional methods of solvent extraction of plant materials are mostly based on the choice of solvents and use of heat and/or agitate to increase the solubility of materials and the rate of mass transfer [7]. Soxhlet extraction involves solid–liquid contact for removal of one or more compounds from a solid by dissolution into a refluxing liquid phase. Its most important advantage is to bring the sample to contact with fresh portions of the solvent repeatedly, which prevents the possibility of the solvent becoming saturated with extractable material and thus enhances the removal of the compounds from the matrix. Heat reflux extraction is a solid–liquid extraction, which is accomplished by allowing hot solvent to leach out the compounds from the solid tissue. This technique allows extraction of the solid at an elevated temperature without loss of solvent under evaporation. Moreover, because the system temperature is slightly higher than the boiling point of the solvent, this excess energy in the form of heat helps to increase the extraction kinetics of the system [8]. Microwave heating depends on the presence of polar molecules or ionic species. Microwave-assisted extraction offers a rapid delivery of energy to the total volume of solvent and subsequent rapid heating. Moreover, when microwave radiation can be focused directly onto the sample, heating is more efficient and thus homogeneity and reproducibility are improved greatly [9], but it is a heat process essentially and has all disadvantages of thermal processing. Ultrasound-assisted extraction enhances both solvent penetration into plant materials and the release of intracellular matter by disruption of the cell walls mainly due to the mechanical effects of acoustic cavitation [7], therefore the optimum condition for the extraction is achieved at higher intensity, higher ratio, and lower temperature, but it is difficult to industrial scale. Supercritical fluid extraction (SFE) [10] has become a focus of interest in the field of extraction from natural materials in the food, pharmaceutical, and biotechnology industries because of the excellent mass transfer properties and ease of control using temperature, pressure or a modifier [11]. CO_2 has been the solvent of choice for most SFE studies primarily because it has a relatively low critical temperature and pressure, low toxicity, relatively high purity, and low cost [12]. However, pure CO_2 frequently fails to extract many organics from a sample matrix efficiently [12] and modifier fluids have been used to increase extraction efficiencies [12]. The modifiers can either increase the solubility of the target analyte or interact with active sites on the sample matrix, which helps CO_2 to extract the analyte efficiently. The most common modifier used in SFE is methanol, ethanol, because of its high solvent polarity, which increases the polarity of CO_2 greatly. The effect of the modifier depends on the modifier, the target analyte, and the sample matrix [13], thus its advantage of easily separation of the solvent and solute is lost obviously.

UPE is a non-thermal processing method that is being looked upon with interest since the higher yield and less impurity, particular qualities of extraction such as nutrient retention, activity and structure are generally not adversely affected by the pressure process. When raising the pressure, the osmotic pressure increases, and the solvent gets into the interior of cells by penetration. The interior of cells will be filled with solvents in a short time. In this process, the volume of the gas, which existed in the capillary and/or cavity of the matrix is greatly decreased, and the solvents penetrated into the inner of the cells very fast. At the same time, the structures of cells were destroyed by some degree. After some time when the intracellular matter got in touch with the solvent, the bioactive components will dissolve in the solvent and the dissolution balance can be reached in a very short time. So extraction time is very short. As pressure released, the pressure in the outer of cells went down to zero suddenly, while the pressure in the inner of cells still remained the value of the balance, which greatly increased the speed at which the component diffused outside. Thus the solubility of the ginsenosides is rapid and complete. UPE is operated at room temperature, many components, such as chlorophyl and gum, will not be dissolved into the solvent. So the extraction liquid has little impurity.

4. Conclusion

Ultrahigh pressure extraction (UPE), as a new extraction method, is used to extract ginsenosides from *P. quinquefolium* L. root. Comparing with other extraction methods, UPE has excel-

lent advantages, such as shorter extraction time, less impurity, higher yield, lower energy consumption, eco-friendly and so on. In the investigated ranges of solvent, pressure and time, the optimization conditions of UPE for *P. quinquefolium* L. root are: solvent is 50% ethanol–water solution, the ratio of solvent to material (ml/g) is 50, extraction pressure is 200 MPa, extraction time is 2 min. UPE is expected to offer a new way for the production and analyses of the plant extractions, and the modernization of pharmaceutical engineering of traditional Chinese herbal medicine.

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