Effects of Extraction Methods on Contents and Compositions of Ginsenosides from Cultivated Jilin Ginseng Extracts

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Abstract：The effect of different extraction methods, supercritical fluid extraction (SFE), ultrasound-assisted extraction and heat reflux extraction, on the extraction efficiencies and compositions of 6 major ginsenosides including Rg1, Re, Rb1, Rc, Rb2 and Rd from cultivated Jilin ginseng was investigated. The ginsenosides were determined by HPLC. The total yield of ginsenosides extracted by SFE, ultrasound-assisted extraction and heat reflux extraction was respectively 0.8557%, 2.2938% and 2.4804%, and the yields of Rg1, Re, Rb1, Rc, Rb2 and Rd were 0.1287%, 0.1169%, 0.2830%, 0.1090%, 0.1061% and 0.1120% when using SFE, 0.3892%, 0.3414%, 0.8088%, 0.2932%, 0.3180% and 0.1432% when using ultrasound-assisted extraction, and 0.3914%, 0.3396%, 0.8898%, 0.3300%, 0.3620% and 0.1676% when using heat reflux extraction, respectively. In the HPLC chromatogram of the heat reflux extract, several peaks disappeared, suggesting the degradation of malonyl ginsenoside. In addition to six common gensinosides, a small amount of unknown secondary ginsenosides were also detected in the extracts from three methods. Based on this, we deduced that neutral ginsenosides were degraded to different extents under SFE, ultrasound assisted-extraction and heat reflux extraction. The above results showed that there was a significant difference in the extract yields and compositions of ginsenosides when different extraction methods were employed. In respect to SFE, the extraction yields of ginsenosides were significantly lower than those observed when using ultrasound-assisted extraction and heat reflux extraction. However, SFE had excellent advantages such as simpler separation process, the absence of solvent contamination and better thermo-sensitive substance protection and higher re-utilization value of the remaining residue.

Key words：ginsenosides；high performance liquid chromatography (HPLC)；extraction methods；extract

提取方法对吉林种植人参提取物皂苷含量及组成的影响

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摘 要：采用超临界CO_2萃取法(SFE)、超声波辅助提取法和溶剂回流法提取吉林种植人参中的人参皂苷；以人参中6种主要人参皂苷Rg1、Re、Rb1、Rc、Rb2、Rd的提取率为指标，采用高效液相色谱法(HPLC)进行测定，考察不同提取方法所得提取液中6种主要人参皂苷提取产率和组成的差异。3种提取液中均检测出了6种常见的人参皂苷Rg1、Re、Rb1、Rc、Rb2、Rd，其提取率之和：超临界CO_2萃取法为0.8557%，人参皂苷Rg1、Re、Rb1、Rc、Rb2、Rd的提取率分别为0.1287%、0.1169%、0.2830%、0.1090%、0.1061%、0.1120%；超声波辅助提取法为2.2938%，人参皂苷Rg1、Re、Rb1、Rc、Rb2、Rd分别为 0.3892%、0.3414%、0.8088%、0.2932%、0.3180%、0.1432%；回流法提取为2.4804%，人参皂苷Rg1、Re、Rb1、Rc、Rb2、Rd分别为0.3914%、0.3396%、0.8898%、0.3300%、0.3620%、0.1676%。回流提取液的HPLC色谱图中某些峰消失，表明丙二酰基人参皂苷发生了降解。除了6种常见人参皂苷外，在3种萃取液中还检测出少量的未知峰，可以推断出在SFE、超声提取和回流提取条件下，中性皂苷发生了不同程度的降解。实验结果表明，提取方法不同，人参皂苷的提取率及组成具有较大差异，超临界CO_2萃取法人参皂苷得率低于超声波辅助提取法和回流提取法，但较其他方法其具有分离工艺简单、无溶剂污染以及保护热敏性物质、萃余物再利用价值高等优势。

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Ginseng, the root of *Panax ginseng* C.A. Meyer, is known as one of the most common Chinese traditional herbs and is used for the treatment of various disease for thousands of years[1]. It is cultivated mainly in northeast China, such as Jilin and Liaoning province, but also grows very well in other regions in the world[2]. The most thoroughly investigated active components of ginseng are known as ginsenosides, a homologous series of triterpenoid saponins with differing glycosylation patterns[3], which account for 4% of the dry weight of ginseng root[4]. Ginsenosides have been reported to have numerous medicinal benefits, including anti-tumour, chemopreventive, immunomodulating and anti-diabetic activities[5]. More than 40 ginsenoside monomers have been reported from ginseng by now[6]. The contents of ginsenoside Rg1, Re, Rb1, Rc, Rb2 and Rd are the most abundant, accounting for 70% of the total ginsenosides, which are commonly used as main index for ginseng product evaluation and ginseng indentification[7]. There are many extraction techniques for ginsenosides, in which the conventional reflux extraction with solvent is commonly used nowadays. In recent years, several auxiliary extraction techniques such as ultrasound-assisted extraction and microwave-assisted extraction have been applied in the ginsenosides extraction. However, the application of supercritical fluid extraction in ginsenoride extraction have barely been reported. Given the small amount of literature devoted to SFE of ginsenorides, and in particular the artificially cultivated Jilin ginseng, this research intended to study the effect of different extraction methods (including SFE, ultrasound-assisted extraction and microwave-assisted extraction) on the six main ginsenosides. The extract yields of ginsenosides were used as the index and the determination was carried out by HPLC. This study would provide scientific reference for establishing and optimizing a green extraction process of ginsenosides.

1 Materials and Methods

1.1 Materials and chemicals

Artificially cultivated Jilin ginseng were obtained in Changbai region of Jilin province. It was grounded and passed through 0.84 mm (20-mesh), 0.42 mm (40-mesh) and 0.25 mm (60-mesh) stainless steel sieves. Ginsenoside Rg1, Re, Rb1, Rc, Rb2 and Rd, as the standard samples, were purchased from Chinese Medical and Biological Products Institute (Beijing, China). Carbon dioxide Carbon dioxide (99.99% purity) was of food grade and purchased from Changchun oxygen factory. HPLC grade methanol and acetonitrile were obtained from Yuwang Industry Ltd. (Shandong, China).

1.2 Equipments

The SFE apparatus(model HA121-50-02) was from Huaan Supercritical Extraction Co. (Nantong, Jiangsu, China). It comprised primarily a syringe pump, a pre-heater, a 500 mL extraction vessel, two separators and a wet gas meter. The Agilent 1200 controlled by the Chemstation software and equipped with a UV detector was from Agilent Technologies (USA). The ultrasonic cell disruptor (model JY92-II) was from Xinzhi Biotechnology Ltd. (Ningbo, China). The rotary evaporator (model RE-52AA) was from Yarong Biochemistry Instrument Plant (Shanghai, China). The high-speed multifunction grinder (model Q-250A_3) was from Shuidu Electrical Appliance Ltd. (Shanghai, China).

1.3 Preparation of samples

1.3.1 Supercritical fluid extraction (SFE)

100 g of Ginseng powder (40－60 mesh) was added with 100 mL of modifier and the mixture was allowed to equilibrate at 25 ℃ for 10 to 12 hours before loaded into an extractor. According to the methods of Liu Chunming et al[8] and Zhang Le et al[9], 70% (*V*/*V*) ethanol-water was used as the modifier. Ginsenosides extraction was performed when extraction pressure was set at 30 MPa, temperature was set at 45 ℃, extraction time was set at 3 hours, CO_2 flow rate set at 12 L/h, temperature and pressure of separator Ⅰ were set at 35 ℃ and 6.2 MPa, and temperature and pressure of separator Ⅱ were set at 30 ℃ and 6.2 MPa. The extract was evaporated to dryness using a rotary evaporator at reduced pressure and the solvent recovered could be reused. When the residue was dissolved in methanol, it was transferred to a 100 mL volumetric flask. Finally, the volume was made up to the mark with methanol 1.5 mL of solution was evaporated to dryness and the resulting residue was dissolved with 30 mL of distilled water. Then the aqueous solution was transferred to a separating funnel and extracted three times with 30 mL of water-saturated *n*-butyl alcohol each time. After the *n*-butyl

alcohol fraction was collected, it was evaporated to dryness by rotary evaporation and the residue was dissolved with methanol once again. The solution was transferred to a 25 mL volumetric flask and the volume was made up to the mark with methanol. The sample solutions for analysis was finally obtained after the solution was diluted in methanol by six-fold and filtered through a 0.22 μm membrance.

1.3.2 Heat reflux extraction

The ginsenosides were extracted with conventional heat reflux extraction according to the optimized conditions reported by Kim et al[10]. 1 g of Ginseng powder(40－60 mesh) was mixed with 50 mL of 70% (*V*/*V*) ethanol-water in a 150 mL round bottom flask fitted with a cooling condenser. The flask was incubated in a water bath at 80 ℃ for 4 hours. When the extraction was completed, the extract was filtered and the residue was rinsed three times with the extraction solvent. Both filtrate and the solvent used for rinsing were collected and transferred to a rotary evaporator. Ethanol was recovered at reduced pressure and the residue was dissolved in 30 mL of distilled water. Then the aqueous solution was transferred to a separating funnel and extracted three times with 30 mL of water-saturated *n*-butyl alcohol each time. Then the *n*-butyl alcohol fraction was collected and evaporated to dryness through rotary evaporation. The residue was dissolved with methanol and transferred to a 10 mL volumetric flask. Finally, the volume was made up to the mark with methanol. The sample solutions for HPLC analysis was obtained after the solution was diluted 6 times and filtered through a 0.22 μm nylon membrance .

1.3.3 Ultrasound-assisted extraction

The ginsenosides were extracted with ultrasound-assisted extraction according to the method developed by Chen Ruizhan[11]. 1 g of Ginseng powder(40－60 mesh) was mixed with 50 mL of 70% (*V*/*V*) ethanol-water and the mixture was transferred to a 250 mL flask, placed in an ultrasonic cleaner. Ultrasonification was carried out for 40 min at room temperature when working time was set at 6 s, interval time was set at 8 s and output power was 200 W. When the extraction was completed, the resulting extract was filtered and collected. The filtrate was evaporated to dryness by a rotary evaporator under reduced pressure, during which ethanol was recovered. The residue was dissolved in 30 mL of distilled water and transferred to a separating funnel. Then the aqueous solution was extracted three times with 30 mL of water-saturated *n*-butyl alcohol each time. Then the *n*-butyl alcohol fraction was collected and evaporated to dryness through rotary evaporation. The residue was dissolved with methanol and transferred to a 10 mL volumetric flask and the volume was made up to the mark with methanol. The sample solution was diluted in methanol 6 times and filtered through a 0.22 μm nylon membrance before HPLC analysis.

1.4 HPLC analysis of ginsenoside monomers

1.4.1 Condition of HPLC

The contents and components of ginsenosides were determined by a Agilent 1200 liquid chromatograph. Chromatography was conducted with a reverse phase Agilent ZORBAXSB-C_{18} column and the temperature of column was controlled at 30 ℃. The number of theoretical plates was no less than 6000. The binary gradient elution solvent consisted of acetonitrile (A) and water (B). A gradient elution program was used: 0 min, 15% A, 85% B; 10 min, 19% A, 81% B; 26 min, 23% A, 77% B; 37 min, 30% A, 70%B; 45 min, 36% A, 64% B; 50 min，45% A, 55% B; 58 min, 65% A, 35% B; 66 min, 80% A, 20% B; 72 min, 100% A; 80 min; 100% A. The flow rate of the mobile phase was maintained at a constant 1.0 mL/min, and the absorbance was measured at a wavelength of 203 nm for the detection of ginsenosides.

1.4.2 Preparation of standard solutions

Standard stock solutions of six ginsenosides Rg1, Re, Rb1, Rc, Rb2 and Rd were prepared by dissolving measured quantities of standard ginsenosides in HPLC grade methanol. The concentrations of the stock solutions of ginsenoside Rg1, Re, Rb1, Rc, Rb2 and Rd were 1.02, 1.03, 1.00, 0.99, 1.03, 0.97 mg/mL respectively. 167 μL of standard stock solutions of ginsenoside Rg1, Re, Rb1, Rc, Rb2, and Rd were mixed together to prepare a mixed standard solution .

1.4.3 Investigation of linearity

Table 1 Regression equations of ginsenoside monomers

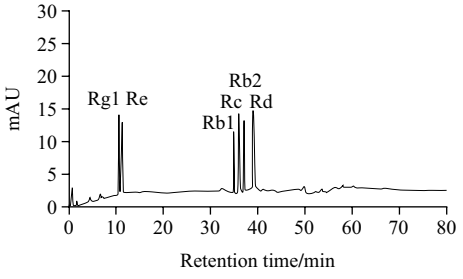
Ginsenoside	Regression equation	Correlation coefficient (r^2)	Range of linearity/μg
Rg1	Y=454.66X+2.5938	0.9998	0.085—2.720
Re	Y=427X−0.4602	1	0.086—2.752
Rb1	Y=307.52X−2.6723	0.9999	0.083—2.656
Rc	Y=417.67X−9.0415	0.9997	0.082—2.624
Rb2	Y=325.21X−1.6106	1	0.086—2.752
Rd	Y=375.2X−2.1469	1	0.081—2.592

0.5, 1, 2, 4, 8 μL and 16 μL of mixed standard solution were injected into the HPLC system respectively. HPLC analysis was carried out under the above-mentioned conditions. The standard curve was constructed via a linear regression of the peak area (*Y*-axis) and the injection quantity (μg) of standard ginsenoside (*X*-axis). The linear regression equations of ginsenosides were listed in Table1.

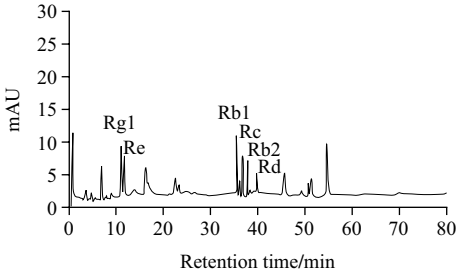
1.4.4 Determination of samples

Sample solutions for analysis were prepared according to 1.3.1. A 5 μL volume of the filtered sample was injected into the HPLC system in triplicate. Based on the regression equations, the contents of the six ginsenosides were calculated by corresponding peak areas. Comparison of the extract yields of ginsenoside Rg1, Re, Rb1, Rc, Rb2, Rd obtained by SFE, heat reflux extraction and ultrasound-assisted extraction was carried out. One-way analysis of variance (ANOVA) was applied to analyze the extract yields of ginsenosides and Student Newman-Keuls (S-N-K) test was performed for the multiple comparison of the extract yields of ginsenosides obtained by different extraction methods using SPSS 16.0 (SPSS Inc., Chicago, USA).

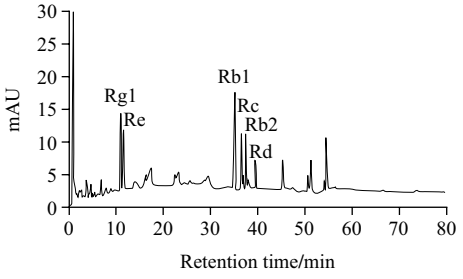
2 Results and Discussion



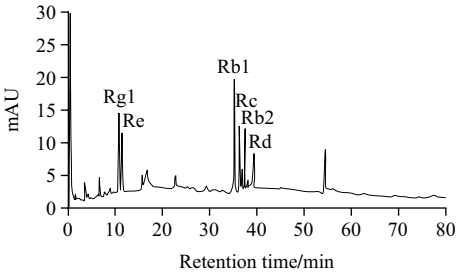
A. HPLC Chromatogram of standard ginsenoside



B. HPLC chromatogram of extract obtained by SFE



C. HPLC chromatogram of extract obtained by ultrasound-assisted extraction



D. HPLC chromatogram of extract obtained by heat reflux extraction

Fig.1 HPLC chromatograms of ginsenosides in extracts obtained by different methods

Table 2 Comparison of extract yields and compositions of six ginsenosides obtained by different extraction methods

%

Extraction methods	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
SFE	0.1287±0.0144^{a}	0.1169±0.0072^{a}	0.2830±0.0118^{a}	0.1090±0.0062^{a}	0.1061±0.0031^{a}	0.1120±0.0194^{a}	0.8557±0.0236^{c}
Ultrasound-assisted extraction	0.3892±0.0239^{b}	0.3414±0.0255^{b}	0.8088±0.0657^{b}	0.2932±0.0208^{b}	0.3180±0.0177^{b}	0.1432±0.0211^{ab}	2.2938±0.1175^{b}
Heat reflux extraction	0.3914±0.0263^{b}	0.3396±0.0197^{b}	0.8898±0.1162^{b}	0.3300±0.0319^{b}	0.3620±0.0299^{c}	0.1676±0.0242^{b}	2.4804±0.1836^{b}

Note: Values were expressed as 'mean value ± SD' (n=3); Student Newman-Keuls (S-N-K) test was performed for the multiple comparison of the extract yields of ginsenosides obtained by different extraction methods and the results were marked with superscript lowercase letters ($^{a-c}$); Values bearing different superscript lowercase letters in the same column are significantly different ($P < 0.05$), and those bearing any of the same superscript lowercase letters are insignificantly different ($P > 0.05$).

SFE, ultrasound-assisted extraction and heat reflux extraction were applied to extract the ginsenosides from artificially cultivated Jilin ginseng and the extract yields of six ginsenoside Rg1, Re, Rb1, Rc, Rb2 and Rd obtained by the three methods were compared. As shown in Table 2, the extract yields of the six ginsenosies for heat reflux extraction were highest, followed by those obtained by ultrasound-assisted extraction. However, in respect to the total yield of the six ginsenosides, not significant difference ($P>0.05$) was found between heat reflux extraction and ultrasound-assisted extraction. Regarding SFE, the totall yield was found to be the lowest, which was about 35% of those obtained by the other two methods, and the extract yields of the six ginsenosides was significantly lower ($P<0.05$) than those of the other two methods. In respect to the three extraction methods, it was samely found that the extract yield of ginsenoside Rb1 was relatively higher, followed by that of ginsenoside Rg1 and Re, and the extract yields of ginsenoside Rg1, Re and Rb1 accounted for 65% of the total yield. The reported pharmacological activities of ginsenoside Rb1 include improvement of memory, central nervous system suppression[12], easing pain, sleep induction, and nervous stabilization[13]. Ginsenoside Rg1 is known to have effects of promoting central nervous excitation, anti-fatigue and

improvement of biosynthesis of DNA and RNA[14], while ginsenoside Re has been demonstrated to have functions of central nervous system suppression and promotion of adrenocorticotrophic hormone secretion[15].

The chromatograms of ginseng root extract obtained by the three extraction methods are shown in Fig. 1B, C, D. By comparing retention times of unknown peaks with mixed standards(Fig. 1A), six main ginsenorides including Rg1, Re, Rb1, Rc, Rb2 and Rd, were all indentified in the extract obtained by SFE, ultrasound-assisted extraction and heat reflux extraction. By comparing chromatograms of the three extraction methods, it was observed that with respect to heat reflux extraction, Rb1, Rc, Rb2 and Rd peaks were obviously higher than the corresponding peaks of the other two extraction methods, and peaks between 42 min and 52 min disappeared(Fig. 1D). In addition to these common ginsenosides, four acidic ginsenosides, termed malonyl ginsenosides, were also known to be present in significant quantities in ginseng[16]. However, these ginsenosides were thermally unstable and were not observed in analysis of the heat reflux extract. Many studies have been done on the degradation of malonyl ginsenosides applying various methods. Wang Yutang et al[17] reported that during long time thermal extraction, the malonyl ginsenosides m-Rb1, m-Rc, m-Rb2 and m-Rd were much less stable than the corresponding neutral ginsenosides and degraded into corresponding neutral ginsenoside Rb1, Rc, Rb2 and Rd. Therefore the malonyl ginsenosides disappeared after heat reflux extraction while the corresponding neutral ginsenosides increased. Except for the six common ginsenosides, unknown peaks were also found at 6, 16, 23 min and 54 min respectively with respect to the extract obtained by the three methods(Fig. 1B, C, D). These unknown peaks were probably secondary ginsenosides or rare ginsenosides. These ginsenosides, including Rh1, Rg3, Rg2, Rg5, Rk1 etc., are not naturally present in ginseng but in the degradation products of ginsenosides[18]. Wang Yutang et al[19] reported that degradation of abundant neutral ginsenosides occurred in high pressure or under high temperature, producing the corresponding C_{20}-deglycosyl secondary ginsenosides. Rare ginsenosides is valued for pharmaceutical use. Ginsenoside Rg3, for example, have functions of inhibiting tumor cell proliferation and resisting tumorous cellular infiltration and tumor metastasis[20]. Ginsenoside Rh1 is also demonstrated to be against cancer[21].

The extract yields of various ginsenoside monomers obtained by different extraction methods were different to some extent. Thermal reflux extraction could effectively promote the solubility and diffusion of bioactive constituents[22], therefore maximizing the extract yields of ginsenosides. It is an effective method for extracting small amount of ginsenosides on an laboratory-scale[23]. Ultrasound-assisted extraction utilises acoustic cavitation and mechanical vibration to increase molecular movement frequency and penetrating power of solvent[24], offering advantages like improved efficiency, reduced extraction time and lowered extraction temperature[25]. SFE is more appropriate for the extraction of lipophilic and small molecular substances, but for the large molecular and polar substances SFE exhibits a low extract yield[26]. Comparing with other extraction methods, SFE has excellent advantages, such as simple technological process, saving labors and lots of organic solvent, and reducing extraction temperature[27]. What's more, a large quantities of byproduct is co-produced when SFE is applied for the manufacture of ginsenosides, which is free of solvent contamination and therefore could be used as the ingredient for other processed ginseng products.

3 Conclusions

SFE, ultrasound-assisted extraction and heat reflux extraction were applied to extract ginsenosides from artificially cultivated Jilin ginseng. The results indicated that different extraction methods had a direct influence on the content and component of ginsenosides. Six common ginsenosides extracted by SFE were lower than those extracted by ultrasound-assisted and heat reflux extraction methods. Although the extract yields for heat reflux and ultrasound-assisted extractions were found superior compared to SFE, heat reflux extraction is time consuming and needs high extraction temperature. What's more, a large amount of organic solvent used in heat reflux extraction may cause environmental and solvent recycling problems, which drives the industrial costs. Ultrasound-assisted extraction also causes the problem of noise pollution. And because the sound wave decays quickly in solvent, it would be hard to be applied to commercial process. SFE is high selective extration and because its separation process is simplified, and the solvent recycling apparatus is not needed. SEF is particularly suited for obtaining natural thermo sensitive constitutes and the products do not present residues of organic solvents. Therefore, all of the advantages that SEF

has could make up for its relatively low yield. Through comparison of three extraction methods, it can be concluded that SFE is a suitable method for the industrial production of ginsenosides considering its numerous potential advantages over conventional extraction processes.

References:

[1] TIAN Meihong, WEI Min, ZHANG Liping, et al. Structure analysis of pectin SB1-1 from the root of *Panax ginseng*[J]. Chemical Research in Chinese Universities, 2005, 21(3): 306-308.

[2] YOU Zongfang. Studies on quality evaluation of under forest ginseng[D]. Changchun: Jilin Agricultural University, 2007.

[3] JUNG M J, JEON B S, BOCK J Y. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (*Panax ginseng* C.A. Meyer)[J]. Food Chemistry, 2002, 79(1): 105-111.

[4] SHI Wei, WANG Yutang, LI Juan, et al. Investigation of ginsenosides in different parts and ages of *Panax ginseng*[J]. Food Chemistry, 2007, 102(3): 664-668.

[5] KWON J H, BÉLANGER J M R, PARÉ J R J, et al. Application of the microwave-assisted process (MAPTM^{TM}) to the fast extraction of ginseng saponins[J]. Food Research International, 2003, 36(5): 491-498.

[6] SHI Wei. The studies on chemical constituents and pesticide residues in different parts and growth periods of *Panax ginseng* C. A. Mey[D]. Changchun: Jilin University, 2007.

[7] LEE S I, KWON H J, LEE Y M, et al. Simultaneous analysis method for polar and non-polar ginsenosides in redginseng by reversed-phase HPLC-PAD[J]. Journal of Pharmacrutical and Biomedical Analysis, 2012, 60(23): 80-86.

[8] LIU Chunming, ZHAO Xueliang, LI Hongmei, et al. Supercritical fluid extraction of saponins from ginseng[J]. Chemical Research in Chinese Universities, 2005, 21(6): 757-760.

[9] ZHANG Le, SONG Fengrui, WANG Qi, et al. Supercritical CO_2 extracting of rare ginsenosides[J]. Chinese Journal of Applied Chemistry, 2010, 27(12): 1483-1485.

[10] KIM S J, MURTHY H N, HAHN E J, et al. Parameters affecting the extraction of ginsenosides from the adventitious roots of ginseng (*Panax ginseng* C.A. Meyer)[J]. Separation and Purification Technology, 2007, 56(3): 401-406.

[11] CHEN Ruizhan. Study on the process and mechanisim of extraction ginsenoside by ultrahigh pressure technology[D]. Changchun: Jilin Universities, 2005.

[12] YANG Qin, CHEN Yunbo, RUI Fuman, et al. Effects of ginsenoside Rb1 on mechanism of large-conductance calciumactivated potassium channels in alzheimer's disease model cells[J]. Journal of Guangzhou University Traditional Chinese Medicine, 2010, 27(6): 599-605.

[13] YUAN Q L, YANG C X, XU P, et al. Neuroprotective effects of ginsenoside Rb1 on transientcerebral ischemia in rats[J]. Brain Res, 2007, 1167(5): 1-12.

[14] ZHANG Minghua, FAN Junming, XIE Xisheng, et al. Ginsenoside-Rg1 protects podocytes from complement mediated injury[J]. Journal of Ethnopharmacolgy, 2011, 137(1): 99-107.

[15] JANG S E, JUNG I H, JOH E H, et al. Antibiotics attenuate anti-scratching behavioral effect of ginsenoside Re in mice[J]. Journal of Ethnopharmacology, 2012, 26(1): 105-112.

[16] LI Xuwen. Studies on ginsenoside degradation and chemical constituents of degradation products[D]. Changchun: Jilin University, 2008.

[17] WANG Yutang, YOU Jingyang, YU Yong, et al. Analysis of ginsenosides in *Panax ginseng* in high pressure microwave-assisted extraction[J]. Food Chemistry, 2008, 110(1): 161-167.

[18] LEAL P F, KFOURI M B, ALEXANDRE F C, et al. Brazilian ginseng extraction via LPSE and SFE: global yields, extraction kinetics, chemical composition and antioxidant activity[J]. Journal of Supercritical Fluids, 2010, 54(1): 38-45.

[19] WANG Yutang, LU Xuwen, JIN Haiyan, et al. Degradation of ginsenosides in root of *Panax ginseng* C. A. Mey by highpressure microwave-assisted extraction[J]. Chemical Research of Chinese Universities, 2007, 28(12): 2264-2269.

[20] PAN X Y, GUO H, HAN J, et al. Ginsenoside Rg3 attenuates cell migration via inhibition of aquaporin 1 expression in PC-3M prostate cancer cells[J]. European Journal of Pharmacology, 2012, 683(1): 27-34.

[21] LEE Y J, JIN Y R, LIM W C, et al. A ginsenoside-Rh1, a component of ginseng saponin, activatesestrogen receptor in human breast carcinoma MCF-7 cells[J]. The Journal of Steroid Biochemistry and Molecular Biology, 2003, 84(4): 463-468.

[22] CHEN Junhui, XIE Mingyong, ZHANG Zhongwei, et al. Determination of ginsenosides in *Panax quinquefolium* L. by HPLC and comparison of different extracting methods[J]. Natural Product Research and Developement, 2006, 18(1): 120-125.

[23] KIM S J, MURTHY H N, HAHN E J, et al. Parameters affecting the extraction of ginsenosides from the adventitious roots of ginseng (*Panax ginseng* C.A. Meyer)[J]. Separation Purification Technology, 2007, 56(3): 401-406.

[24] CHEN Ruizhan, MENG Fanlei, ZHANG Shouqin, et al. Effects of ultrahigh pressure extraction conditions on yields and antioxidant activity of ginsenoside from ginseng[J]. Separation Purification Technology, 2009, 66(2): 340-346.

[25] ZHANG Shouqin, CHEN Ruizhan, WU Hua, et al. Ginsenoside extraction from *Panax quinquefolium* L. (American ginseng) root by using ultrahigh pressure[J]. Journal of Pharmaceutical and Biomedical Analysis, 2006, 41(1): 57-63.

[26] SHU Xiaoshun, GAO Zhonghong, YANG Xiangliang. Supercritical fluid extraction of sapogenins from tubers of *Smilax china*[J]. Fitoterapia, 2004, 75(7): 656-661.

[27] WOOD J A, BERNARDS M A, WAN W K, et al. Extraction of ginsenosides from North American ginseng using modified supercritical carbon dioxide[J]. The Journal of Supercritical Fluids, 2006, 39(1): 40-47.