Journal of Medicinal Plants Research Vol. 6(14), pp. 2812-2815, 16 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1235
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Effects of steaming on chemical composition of *Panax ginseng* hairy roots and *Panax ginseng*

Pei He Zheng, Chang Yu Li, Shi Feng Pang, Yi Ming Guan, Cai Shao, Shi Quan Xu, Ji Yong Liu, Wei Hou, Xiu Juan Lei and Ying Ping Wang*

Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences, Jilin, 132109, China.

Accepted 27 March, 2012

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) hairy root and ginseng (*P.* ginseng C. A. Meyer, Araliaceae) root were steamed at 100°C for 3 h. Thereafter treatment, the content of ginsenosides, Rg1, Re, Ro, Rf, Rb1, Rc, Rb2, Rb3 and Rd decreased. On the other hand, the content of ginsenosides, Rg3, Rg2 and Rh1 increased. Two previously identified anticancer ginsenosides, Rg3 and Rh2 increased significantly in steamed ginseng hairy roots from 0.017 to 1.14 mg/g and not detected to 0.74 mg/g, respectively. In steamed ginseng root, Rg3 increased from 0.0062 to 0.75 mg/g, and Rh2 was not detected. These results indicated that steaming process may provide an effective method for ginseng hairy roots to conversion polar ginsenosides to non-polar ginsenosides Rg3 and Rh2.

Key words: *Panax ginseng,* ginseng hairy roots, steaming, chemical composition, ginsenosides.

INTRODUCTION

Panax ginseng C. A. Meyer (Araliaceae) has been used as a famous and expensive medicine in oriental countries since 2000 years ago (Attele et al., 1999; Lee et al., 2010). Ginseng has a wide range of actions, including, powerfully tonifies primordial qi, restores the pulse and secures abandoned disorders, strengthens the spleen and benefits the lungs, generates fluids and nourishes blood, quiets the spirit and augments intelligence (Commission, 2010). Ginsenosides are the main bioactive components of ginseng (Wu and Zhong, 1999). In Asia, ginseng root is steamed at 100°C to give red ginseng (Takaku et al., 1990). When the red ginseng is contrasted with the white ginseng, the difference is very apparent. The difference in biological effects of red and white ginseng is due to the significant changes in ginsenosides after the steaming treatment (Yun et al., 2001; Nam, 2005; Yoo et al., 2006).

However, it is difficult and time consuming to cultivate ginseng in the field. Thus, many researchers have investigated the production of ginsenosides using callus, cell suspensions and adventitious roots. *Agrobacterium rhizogenes*-mediated hairy roots have been successfully performed in ginseng (Yoshikawa and Furuya, 1987; Yoshimatsu and Yamagwhi, 1996; Zhao et al., 2001). No attempt at steaming *P. ginseng* hairy roots has been reported. In this study, we treated *P. ginseng* hairy roots and ginseng root at 100°C for 3 h to analyse the changes in ginsenosides content.

MATERIALS AND METHODS

The hairy roots of *P. ginseng* C. A. Meyer (Araliaceae) were induced by strain A_4 of *A. rhizogenes* which was obtained from professor Jia Shirong at the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences (Zhao et al., 2001). The hairy roots were cultivated in N/5 Murashige and Skoog (MS) (potassium nitrate and ammonium nitrate were reduced to 1/5) liquid medium for 5 weeks. The fresh five-year-old *P. ginseng* materials were harvested in fall from Fusong county of Jilin province, China. One part of samples was freeze dried by freeze dryer (FDU-2100, Tokyo Rikakikai Co., LTD, Japan) at -80°C for 20 h. The other part of the samples was steamed at 100°C for 3 h, then was dried in forced air drying unit (101-1, Luda Lab apparatus Corporation, China) at different temperatures 65 and 50°C for 12 and 24 h. The dried samples were used for the quantification of ginsenosides. Ginsenosides Rg1, Re, Rf, Rg2, Rh1, Rb1, Rc, Rb2, Rb3, Rd, Rg3 and Rh2 were purchased from Pharmaceutical and Biological Products (Beijing, China). Ginsenoside Ro was isolated from the dried root of *P. ginseng* in our laboratory.

Acetonitrile and methanol were of high performance liquid chromatography (HPLC) grade from Dikma (CA, USA). Other

*Corresponding author. E-mail: yingpingw@126.com. Tel: +86-432-66513091. Fax: +86-432-66513091.

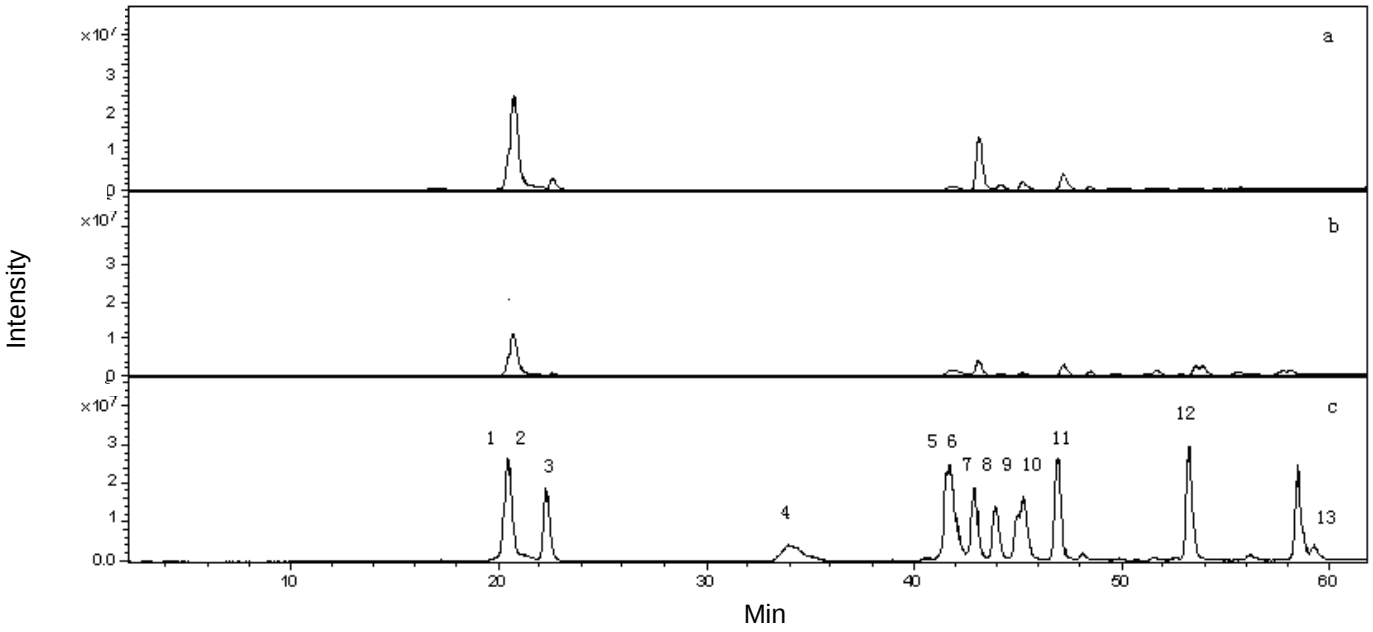

Figure 1. Typical HPLC-MS total ion chromatograms of unsteamed (a), steamed (b) ginseng hairy roots and ginsenoside standards (c). Peaks: (1) Rg1, (2) Re, (3) Ro, (4) Rf, (5) Rh1, (6) Rg2, (7) Rb1, (8) Rc, (9) Rb2, (10) Rb3, (11) Rd, (12) Rg3 and (13) Rh2.

reagents were of analytical grade. Water used in the experiments was generated by a Milli-Q Ultra-pure Water System (Millipore, Billerical, USA).

Instruments operating conditions

An Agilent 1200 liquid chromatographic system (Agilent Technologies, USA) equipped with quaternary pump, a vacuum degasser, an auto sampler, thermostatted column compartment and diode array detector was used. An Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm) was employed to separate ginsenosides in a binary solvent that consisted of 6.5 mM NH_4Ac and 5 mM NH_4OH (A) and acetonitrile (B) with a gradient elution: 0 to 16 min, 17.5 to 21.0% B; 16 to 18.4 min, 21.0 to 26.0% B; 18.4 to 33.6 min, 26.0% B; 33.6 to 44 min, 26.0 to 36.0% B; 44 to 51.2 min, 36.0 to 50.0% B; 51.2 to 58.4 min, 50.0 to 68.0% B; 58.4 to 62 min, 68.0 to 80.0% B; 62 to 70 min, 80.0 to 88.0% B. The flow-rate was kept at 1.0 ml/min and the sample injection volume was 20 µl. The absorbance was measured at a wavelength of 203 nm for the detection of ginsenosides.

Mass spectrometry was carried out on an Agilent Ion Trap MS (Agilent Corp, USA) with the electrospray ionization (ESI) ion source. The conditions of ESI were as follow: negative ion mode, drying gas (N2), 7.0 L/min; temperature, 350°C; nebulizer, 25 psi; scan range m/z of 200 to 1200.

Preparation of standards and samples solutions

Ginsenosides Rg1, Re, Ro, Rf, Rg2, Rh1, Rb1, Rc, Rb2, Rb3, Rd, Rg3 and Rh2 mixed standards solution was prepared in methanol. 1.0 g dried ginseng sample was extracted three times with 10 ml methanol by ultrasonication for 30 min, and then centrifuged at 12000 rpm for 10 min, and 10 µl of the filtrate was injected into the high performance liquid chromatography-mass spectrometry (HPLC-MS).

Method validation

The limit of detection and quantitation (LOD and LOQ) for each gensenoside were measured at a signal-to-noise ratio of 3 and 10 as criteria. Based on the LOQ level, concentration scales and calibration curves were established (regression coefficient between 0.993884 and 0.999981). Intra-day and inter-day variations were chosen to assess the precision of the HPLC-MS method. The intra-day precision was evaluated by triplicate extraction and analysis over the course of a single day. Inter-day variation was performed on three different days. Variations were expressed by the relative standard deviations (RSD). The intra-day and inter-day variations were 4.51 and 4.78% for all analyses. Repeatability and stability were also measured by the RSD, and the overall repeatability and stability variations were less than 4.46 and 4.87%. Recovery test was used to assay the accuracy of this quantification method. By spiking accurate amounts of standards to 1.0 g of the sample, the recovery experiments were performed. This method had good accuracy with overall recovery in the range of 90.9 to 108.9% and RSDs below 5.30%.

RESULTS

Effects of steaming on ginseng hairy roots constituents

Typical HPLC-MS total ion chromatograms of unsteamed, steamed ginseng hairy roots and ginsenoside standards are as shown in Figure 1. Compared with unsteamed ginseng hairy roots, in ginseng hairy roots steamed at 100°C for 3 h, the total ginsenoside contents decreased from 62.00 to 38.49 mg/g. Among the saponins, ginsenoside Rb1 decreased from 11.77 to 3.53 mg/g, ginsenoside Rg1 decreased from 15.95 to 10.73 mg/g,

Table 1. Saponin content in the steamed ginseng hairy roots and ginseng root (mean ± SD; n=3).

Ginsenosides	Ginseng hairy roots		Ginseng root	
	Unsteamed	Steamed	Unsteamed	Steamed
Rg1	15.95 ± 0.36	10.73 ± 0.27	30.62 ± 0.51	15.28 ± 0.33
Re	8.24 ± 0.22	3.48 ± 0.10	1.88 ± 0.14	1.77 ± 0.12
Ro	1.90 ± 0.12	0.57 ± 0.09	5.72 ± 0.17	2.69 ± 0.12
Rf	0.98 ± 0.14	0.87 ± 0.13	2.30 ± 0.09	2.56 ± 0.07
Rg2	0.36 ± 0.09	0.79 ± 0.12	0.34 ± 0.02	0.95 ± 0.05
Rh1	2.58 ± 0.17	2. 90 ± 0.13	2.58 ± 0.11	3.21 ± 0.10
Rb1	11.77 ± 0.28	3.53 ± 0.08	10.95 ± 0.25	5.93 ± 0.13
Rc	1.32 ± 0.06	0.94 ± 0.06	4.76 ± 0.20	3.30 ± 0.18
Rb2	13.14 ± 0.55	8. 45 ± 0.17	35.41 ± 0. 83	27.06 ± 0.41
Rb3	0.72 ± 0.07	0.41 ± 0.05	0.75 ± 0.06	0.74 ± 0.09
Rd	5.01 ± 0.11	3.96 ± 0.14	3.15 ± 0.17	2.13 ± 0.17
Rg3	0.017 ± 0.003	1.14 ± 0.21	0.0062 ± 0.001	0.75 ± 0.11
Rh2	N.D	0.74 ± 0.08	N.D	N.D
Total	62.00	38.49	98.46	66.36

Values are expressed as mg/g of dry weight; N.D., not detected.

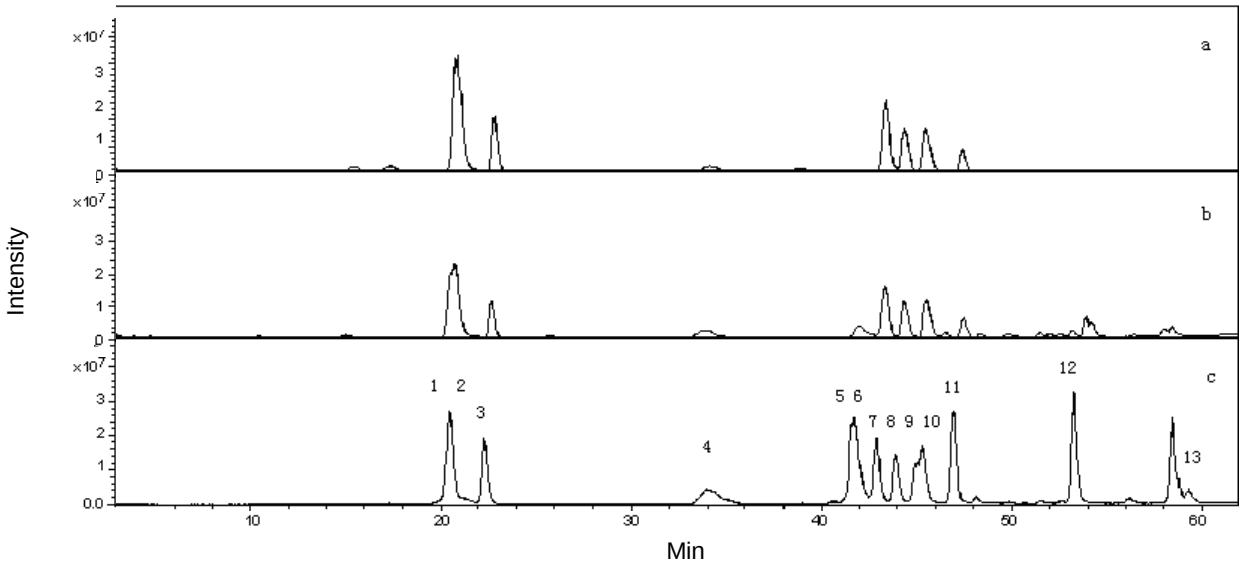

Figure 2. HPLC-MS total ion chromatograms of unsteamed (a), steamed (b) ginseng root and ginsenoside standards (c). Peaks: (1) Rg1, (2) Re, (3) Ro, (4) Rf, (5) Rh1, (6) Rg2, (7) Rb1, (8) Rc, (9) Rb2, (10) Rb3, (11) Rd, (12) Rg3 and (13) Rh2.

ginsenoside Re decreased from 8.24 to 3.48 mg/g, ginsenoside Rg3 increased from 0.017 to 1.14 mg/g, ginsenoside Rh2 increased from 0 to 0.74 mg/g and ginsenoside Rg2 increased from 0.36 to 0.79 mg/g. The content of 13 ginsenosides in steamed ginseng hairy roots is as shown in Table 1.

Effects of steaming on ginseng root constituents

HPLC-MS total ion chromatograms of unsteamed, steamed ginseng root and ginsenoside standards are as shown in Figure 2. Compared with unsteamed ginseng, the roots treated at 100°C for 3 h had significantly decreased total ginsenoside content, from 98.46 to 66.36 mg/g. Among the main saponins, ginsenoside Rg1 decreased from 30.62 to 15.28 mg/g, ginsenoside Rb2 decreased from 35.41 to 27.06 mg/g, ginsenoside Rb1 decreased from 10.95 to 5.93 mg/g, ginsenoside Rg3 increased from 0.0062 to 0.75 mg/g, ginsenoside Rh1 increased from 2.58 to 3.21 mg/g and ginsenoside Rg2 increased from 0.34 to 0.95 mg/g. The content of 13

ginsenosides in steamed ginseng root is as shown in Table 1.

DISCUSSION

The changes of saponin content in ginseng hairy roots and ginseng root were similar to those in American ginseng berry and *Panax notoginseng* root (Wang et al., 2006; Sun et al., 2010). After steaming for 3 h, total saponin content was shown to be significantly different, ginsenosides Rg1, Re, Ro, Rf, Rb1, Rc, Rb2, Rb3 and Rd decreased after the steaming process. On the other hand, ginsenosides Rg3, Rh2, Rg2 and Rh1 increased. Compared to unsteamed ginseng root, in ginseng root steamed, the total content of confirmed saponins decreased significantly, and ginsenosides Rg1, Re, Ro, Rf, Rb1, Rc, Rb2, Rb3 and Rd decreased, but ginsenosides Rg3, Rg2 and Rh1 increased. Although, total saponin contents of steamed ginseng hairy roots is less than steamed ginseng root, the ginsenoside Rg3 of ginseng hairy roots is more than steamed ginseng root. At the same time, ginsenoside Rh2, a trace saponin, was detected in steamed ginseng hairy roots (0.74 mg/g). Ginsenoside Rg3 is a steroidal saponin belonging to protopanaxadiol type, which has an aglycone of dammarane skeleton.

Ginsenoside Rg3 was isolated from white ginseng and red ginseng has been shown to inhibit tumor metastasis and the invasion of several tumors (Qian et al., 2005; Zhao et al., 2010). Ginsenoside Rh2, first isolated from red ginseng, is also an active anticancer saponin, Rh2 can reduce the proliferation of a variety of cultured cancer cells and can influence apoptosis (Gu et al., 2009; Li et al., 2011). The work of Nakata et al. (1998) and Popovich and Kitts (2004) showed that Rh2 exerts significantly more potent colorectal cancer cell killing activities than Rg3.

Conclusions

The results of this study demonstrate that there was a significant difference in saponin content after steaming ginseng hairy roots. Steamed ginseng hairy roots markedly augment ginsenoside Rg3 (1.14 mg/g) and Rh2 (0.74 mg/g), which provide an opportunity to develop a new class of anticancer agent.

ACKNOWLEDGEMENTS

This project was financially supported by Program (Ministry of Science and Technology, People's Republic of China, No. 2007BAI38B03) and Science and Technology Development Program (Jilin Province, China, No. 20096040 and No. YYZX201136).

REFERENCES

Attele AS, Wu JA, Yuan CS (1999). Ginseng Pharmacology: multiple constituents and multiple actions. Biochem. Pharmacol., 58: 1685-1693.

China Pharmacopoeia Commission (2010). China Pharmacopoeia 2010. Beijing: China Med. Sci. Press, pp. 8-9.

Gu Y, Wang GJ, Sun JG, Jia YW, Wang W, Xu MJ, Lv T, Zheng YT, Sai Y (2009). Pharmacokinetic characterization of ginsenoside Rh2, an anticancer nutrient from ginseng, in rats and dogs. Food Chem. Toxicol., 9: 2257-2268.

Lee SY, Kim YK, Park NI, Kim CS, Lee CY, Park SU (2010). Chemical constituents and biological activities of the berry of *Panax ginseng*. J. Med. Plants Res., 5: 349-353.

Li BH, Zhao J, Wang CZ, Searle J, He TC, Yuan CS, Du W (2011). Ginsenoside Rh2 induces apoptosis and paraptosis-like cell death in colorectal cancer cells through activation of p53. Cancer Lett., 2: 185-192.

Nakata H, Kikuchi Y, Tode T, Hirata J, Kita T, Ishii K, Kudoh K, Nagata I, Shinomiya N (1998). Inhibitory effects of ginsenoside Rh2 on tumor growth in nude mice bearing human ovarian cancer cells. Jpn. J. Cancer Res., 89: 733-740.

Nam KY (2005). The comparative understanding between red ginseng and white ginsengs, processed ginsengs (Panax ginseng C. A. Meyer). J. Ginseng Res., 1: 1-18.

Popovich DG, Kitts DD (2004). Mechanistic studies on protopanaxadiol, Rh2, and ginseng (*Panax quinquefolius*) extract induced cytotoxicity in intestinal Caco-2 cells. J. Biochem. Mol. Toxicol., 3: 143-149.

Qian T, Cai Z, Wong RN, Mak NK, Jiang ZH (2005). In vivo rat metabolism and pharmacokinetic studies of ginsenoside Rg3. J. Chromatogr. B., 816: 223-232.

Sun S, Wang CZ, Tong R, Li XL, Fishbein A, Wang Q, He TC, Du W, Yuan CS (2010). Effects of steaming the root of *Panax notoginseng* on chemical composition and anticancer activities. Food Chem., 118: 307-314.

Takaku T, Kameda K, Matsuura Y, Sekiya K, Okuda H (1990). Studies on insulin-like substances in Korean red ginseng. Planta Med., 56: 27-30.

Wang CZ, Zhang B, Song WX, Wang A, Ni M, Luo X, Aung HH, Xie JT, Tong R, He TC, Yuan SC (2006). Steamed American ginseng berry: Ginsenoside analyses and anticancer activities. J. Agric. Food Chem., 26: 9936-9942.

Wu J, Zhong JJ (1999). Production of ginseng and its bioactive components in plant tissue culture: Current technological and applied aspects. J. Biotechnol., 68: 89-99.

Yoo HH, Yokozawa T, Satoh A, Kang KS, Kim HY (2006). Effects of ginseng on the proliferation of human lung fibroblasts. Am. J. Chin. Med., 34: 137-146.

Yoshikawa T, Furuya TT (1987). Saponim production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogenes*. Plant Cell Rep., 6: 447-453.

Yoshimatsu K, Yamagwhi H (1996). Traits of Panax Ginseng hairy roots after cold storage and cryopreservation. Plant Cell Rep., 15: 555-560.

Yun TK, Lee YS, Lee YH, Kim SI, Yun HY (2001). Anticarcinogenic effect of *Panax ginseng* C.A. Meyer and identification of active compounds. J. Korean Med. Sci., 16: S6-S18.

Zhao Q, Zheng X, Jiang J, Zhou H, Hu P (2010). Determination of ginsenoside Rg3 in human plasma and urine by high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr., B. 878: 2266-2273.

Zhao SJ, Yang ZT, LI CY, Zang P, Shen YH (2001). Formation of ginseng hairy roots mediated by *Agrobacterium rhizogenes* and analysis of ginsenosides in hairy roots in vitro. J. Jilin Agric. Univ., 2: 57-63.