

Changes in the Contents of Prosapogenin in the Red Ginseng (*Panax ginseng*) Depending on Steaming Batches

Sun A Lee¹, Hee Kyung Jo¹, Byung Ok Im², Sungun Kim³, Wan Kyun Whang³, and Sung Kwon Ko^{1*}

¹Department of Oriental Medical Food & Nutrition, Semyung University, Jecheon 390-711, Korea

²Department of Natural Medicine Resources, Semyung University, Jecheon 390-711, Korea

³College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea

This study compared the contents of ginsenosides depending on steaming conditions of red ginsengs to provide basic information for developing functional foods using red ginsengs. The red ginseng steamed eight times at 98°C ranked atop the amounts of prosapogenins ever detected in red ginsengs (ginsenoside Rg₂, Rg₃, Rg₅, Rg₆, Rh₁, Rh₄, Rk₁, Rk₃, F₁, F₄, 1.15%) among red ginsengs steamed more than twice. When steamed eight times at 98°C, 2.7 times as much prosapogenins such as ginsenosides Rg₂, Rg₃, Rg₅, Rg₆, Rh₁, Rh₄, Rk₁, Rk₃, F₁, and F₄ as those steamed just once at 98°C was collected. In addition, the red ginsengs steamed eight times at 98°C contained more amounting ginsenoside Rg₅ (0.28%) than that in the red ginseng steamed several times at random. Accordingly, it is recommendable that red ginsengs steamed 8 times, which proved to be the optimal steaming condition, be used rather than those steamed 9 times (black ginsengs), in order to develop red ginseng products of high prosapogenin concentration and high functions.

Keywords: *Panax ginseng*, Red ginseng, Black ginseng, Prosapogenin, Steam, Ginsenoside Rg₅

INTRODUCTION

Ginseng (*Panax ginseng*) is listed as a medicinal herb in the first class article of Shennong Benaojing, a typical Chinese herbal dictionary. A special medicinal herb, ginseng, which is sweet in taste and known to warm up the body moderately, is known to help keep the lungs and spleen healthy [1].

Ginseng contains more than 30 different ginseng saponins which display various physiological activities [2,3], polyacetylenes which are known to display antitumor activities on various cancers [4], phenolic compounds featuring antioxidant activities [5], proteins having radioprotective effects on victims of an atomic air raid [6], and acidic polysaccharides featuring immune controlling activities as demonstrated in a mouse model experiment

[7].

The chemical structure of ginsenoside, the main pharmacological component of ginseng, was identified by the Shibata Group of Tokyo University [8].

Ginsenosides are classified into two groups, protopanaxadiols and protopanaxatriols. The main component of the protopanaxadiols is ginsenoside Rb₁ which is known to suppress the superactivity of the nervous system. The main component of the protopanaxatriols is ginsenoside Rg₁ which stimulates the central nervous system and is deeply involved in the adaptogen activity of the ginseng [9].

Red ginseng (*Ginseng Radix rubra*) refers to steamed ginseng while and ginseng (*Ginseng Radix alba*) refers to

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Received 09 Apr. 2011, Revised 08 Oct. 2011, Accepted 08 Oct. 2011

*Corresponding author

E-mail: skko@semyung.ac.kr

Tel: +82-43-649-1433, Fax: +82-43-649-1759

the dried ginseng.

Fine roots that are dried naturally under the sunlight are fine ginseng roots (*Ginseng Radix palba*). Ginsenoside Rg₃ is not found in raw and white ginsengs but generated during the steaming process, and thus particularly found in small amounts in red ginsengs.

Ginsenosides Rg₃ was found to have an anticancer effect on phorbol ester-induced cyclooxygenase-2 expression, activate NF- κ B and suppress tumor [10], help lowering blood pressure by endothelium-dependant relaxation in rat aorta [11]. Rg₃ in methanol extract of heat-processed ginseng was found to have antioxidant and anti-tumor promoting effects [12].

On the other hand, while Samukawa group of Kinki University compared and analyzed ginseng saponins depending on red ginseng steaming conditions but did not analyze trace ingredients in red ginsengs such as ginsenosides F₁, Rg₆, F₄, Rk₃, Rh₄, Rg₅, and Rk₁ [13].

No study on the chemical transformation of ginseng saponin glycosides into the minor prosapogenins (ginsenoside F₁, Rg₆, F₄, Rk₃, Rh₄, Rg₅, Rk₁, the main prosapogenins of black ginseng) of red ginseng by hydrolysing process under various steaming conditions has been conducted to date. This study compared the contents of ginsenoside depending on the steaming conditions of red ginsengs to provide basic information for developing functional foods using red ginsengs.

MATERIALS AND METHODS

Materials for experiments

Fresh ginseng (*Ginseng Radix aquosa*) was purchased in Punggi town, Yeongju city, Gyeongsangbuk Province, Korea, in August, 2009. The acetonitrile and distilled water for HPLC were purchased from Burdick & Jackson (Muskegon, MI, USA). All other chemicals were of an analytical reagent grade. The standards were purchased from Chromadex (St. Santa Ana, CA, USA) and Ambo Institute (Seoul, Korea).

Preparation of samples

Two hundred gram of fresh ginseng was steamed in a household steamer at 98°C for 2 h. After steaming, it was dried in a drying oven (JEIO TECH, FO-600M) at 60°C for 24 h by repeating its process nine times. Each samples (Fig. 1) were refluxed with ethanol for 4 h. The organic solvent was removed and the residue dissolved in 1,000 mL of water and extracted with 300 mL of diethyl ether. The aqueous layer was further extracted three times with 300 mL of water-saturated n-4 butanol. The

n-4 butanol fraction was evaporated and the residue was dissolved in 40 mL of methanol, which was subjected to HPLC determination. The samples were filtered before injection.

Crude saponin preparation

Samples of 8 g each were collected, residual fluids extracted from ethyl alcohol were concentrated with lowered pressure and processed with diethyl ether three times. Then, they were processed with n-butanol (n-BuOH) three times after fat-soluble substances were removed. The n-BuOH layer thus obtained was extracted with lowered pressure. The content of extracts with lowered pressure was considered to be the quantity of crude saponin [8].

HPLC analysis

Ginsenoside composition of the concentrate was analyzed by HPLC as suggested by Ko *et al.* [14]. The total ginsenoside content and ginsenoside composition of each sample were analyzed three times. The pure ginsenoside standards (99% pure) used in this experiment were purchased from Chromadex and Ambo Institute.

The HPLC instrument used for the experiment was Waters 1525 binary HPLC system (Waters, Milford, MA, USA), and the column used was Eurospher 100-5 C18 (3x250 mm; Knauer, Berlin, Germany). The mobile phase was the mixture of acetonitrile (HPLC grade; Sigma-Aldrich Chem Co., St. Louis, MO, USA) and distilled water (HPLC grade, JT Baker). The content of acetonitrile was sequentially increased from 17% to 30% (35 min), 30% to 40% (60 min), 40% to 60% (100 min), 60% to 80% (110 min), 80% to 80% (120 min), 80% to 100% (125 min), 100% to 100% (135 min) and finally adjusted from 100% to 17% (140 min, lasting for 5 min) again. The operating temperature was set at room temperature, and the flow rate at 0.8 mL/min. The elution profile on chromatogram was obtained by using a UV/VIS detector at 203 nm (2487 dual λ absorbance detector, Waters).

RESULTS AND DISCUSSION

This study proposes to examine the differences arising from the pattern of saponin contents and provide basic information on the ginsenoside contents depending on red ginseng steaming conditions by surveying and comparing the distribution of each individual ginsenoside content of five-year-old fresh ginseng roots of the same origin.

Ginseng saponins analyzed include ginsenosides Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁, Rg₂, Rg₃, Rg₆, Rh₁, Rh₄, Rk₁, Rk₃, Rg₅, F₁, and F₄. Samples were directly compared and confirmed against the reference standard through HPLC as shown in Fig. 2, and their averages processed statistically and calculated. Five-year-old fresh ginseng roots were harvested from ginseng cultivating fields in Punggi town, Yeongju city, Gyeongsangbuk Province, Korea, the

country's principal ginseng production locality, processed in Semyung University and their saponin contents were compared and analyzed.

Contents of crude saponins

Red ginsengs steamed 8 times were found to contain the highest crude saponin contents at 8.51%, followed by those steamed 5 times (7.56%), 9 times (7.53%), 6 times (7.19%), 7 times (6.94%), 4 times (6.81%), 3 times (6.42%), twice (5.84%), and those steamed once (5.29%) as shown in Table 1.

Contents of ginsenosides depending on steaming conditions

Contents of prosapogenin (ginsenosides Rg₂, Rg₃, Rg₅,

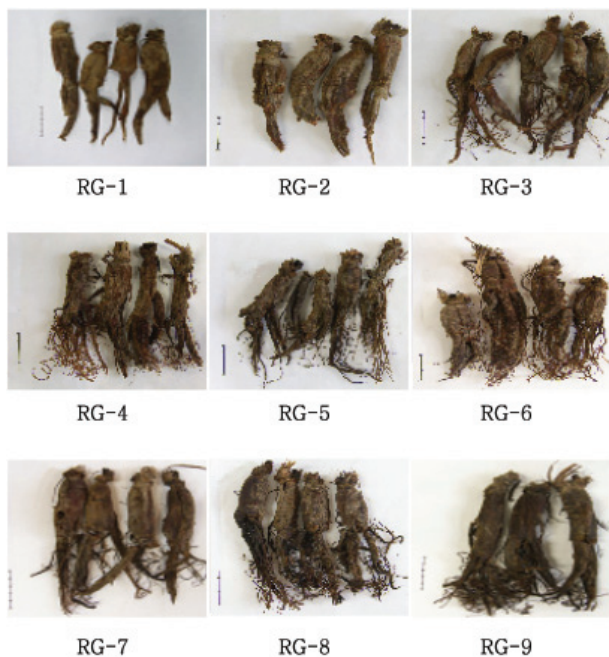


Fig. 1. Red ginseng (RG) portfolio.

Table 1. Contents of crude saponins in red ginseng

Samples	Composition of crude saponins
RG-1 ¹⁾	5.29
RG-2	5.84
RG-3	6.42
RG-4	6.81
RG-5	7.56
RG-6	7.19
RG-7	6.94
RG-8	8.51
RG-9	7.53

Values are presented as percentage.

RG, red ginseng.

¹⁾No. of times being steamed.

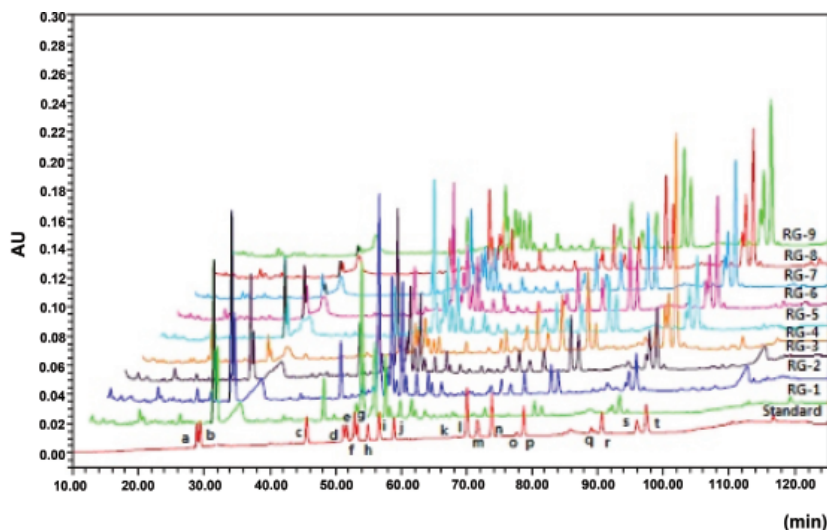


Fig. 2. HPLC chromatogram of ginsenosides detected from the red ginseng (RG) as compared with the chromatogram of the ginsenoside standards. a, ginsenoside Rg; b, ginsenoside Re; c, ginsenoside Rf; d, ginsenoside Rb₁; e, ginsenoside Rg₂; f, ginsenoside Rh₁; g, ginsenoside Rc; h, ginsenoside Rb₂; i, ginsenoside F₁; j, ginsenoside Rd; k, ginsenoside Rg₆; l, ginsenoside F₄; m, ginsenoside Rk₃; n, ginsenoside Rh₄; o, (20S) ginsenoside Rg₃; p, (20R) ginsenoside Rg₃; q, ginsenoside Rk₁; r, ginsenoside Rg₅.

Table 2. Composition of ginsenosides in the red ginseng extracts processed under differing steam conditions

Ginsenosides	Red ginseng (% w/w)								
	RG-1 ¹⁾	RG-2	RG-3	RG-4	RG-5	RG-6	RG-7	RG-8	RG-9
Rb ₁	0.03±0.00	0.03±0.02	0.05±0.04	0.01±0.00	0.02±0.00	0.05±0.01	0.02±0.00	0.05±0.00	0.02±0.00
Rb ₂	0.14±0.01	0.28±0.02	0.21±0.02	0.05±0.00	0.25±0.02	0.17±0.01	0.15±0.02	0.16±0.01	0.13±0.02
Rc	0.22±0.01	0.29±0.03	0.24±0.03	0.04±0.00	0.27±0.03	0.26±0.05	0.15±0.01	0.21±0.03	0.13±0.02
Rd	0.03±0.01	0.04±0.01	0.04±0.01	0.03±0.00	0.05±0.00	0.05±0.00	0.04±0.01	0.06±0.00	0.04±0.01
Re	0.10±0.01	0.12±0.02	0.07±0.01	0.02±0.00	0.07±0.01	0.05±0.00	0.02±0.00	0.03±0.01	0.01±0.00
Rf	0.05±0.00	0.08±0.01	0.08±0.01	0.05±0.00	0.10±0.01	0.09±0.00	0.06±0.01	0.08±0.01	0.07±0.01
Rg ₁	0.24±0.02	0.30±0.03	0.18±0.02	0.05±0.00	0.18±0.02	0.11±0.00	0.05±0.01	0.04±0.00	0.02±0.01
Rg ₂	0.35±0.03	0.50±0.04	0.42±0.06	0.08±0.00	0.47±0.03	0.37±0.01	0.25±0.02	0.27±0.01	0.21±0.03
(20S) Rg ₃	0.02±0.00	0.03±0.00	0.07±0.01	0.11±0.00	0.09±0.01	0.10±0.00	0.12±0.02	0.16±0.01	0.16±0.03
(20R) Rg ₃	0.01±0.00	0.02±0.01	0.05±0.01	0.04±0.00	0.06±0.00	0.07±0.00	0.08±0.01	0.11±0.01	0.11±0.02
Rg ₅	0.02±0.00	0.05±0.01	0.09±0.01	0.37±0.01	0.13±0.01	0.20±0.00	0.24±0.03	0.30±0.02	0.29±0.04
Rg ₆	0.00±0.00	0.01±0.00	0.01±0.00	0.03±0.00	0.15±0.00	0.02±0.00	0.02±0.00	0.03±0.00	0.03±0.01
Rh ₁	0.01±0.01	0.03±0.00	0.05±0.01	0.06±0.00	0.07±0.01	0.08±0.02	0.08±0.01	0.10±0.03	0.08±0.01
Rh ₄	0.00±0.00	0.01±0.00	0.01±0.00	0.04±0.00	0.02±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.01
Rk ₁	0.01±0.00	0.01±0.00	0.02±0.00	0.07±0.00	0.03±0.00	0.05±0.00	0.06±0.01	0.07±0.01	0.07±0.01
Rk ₃	0	0.01±0.00	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00
F ₁	0.02±0.00	0.03±0.00	0.02±0.00	0.00±0.00	0.03±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.01±0.00
F ₄	0.01±0.00	0.01±0.00	0.02±0.00	0.05±0.00	0.03±0.00	0.04±0.00	0.04±0.01	0.05±0.00	0.05±0.01
Rg ₃	0.02	0.05	0.11	0.14	0.14	0.17	0.20	0.28	0.27
Rk ₁ +Rg ₅	0.03	0.06	0.11	0.44	0.17	0.24	0.30	0.37	0.35
Prosapogenin ²⁾	0.43	0.71	0.75	0.86	0.96	0.98	0.94	1.15	1.05
Total saponin ³⁾	1.23	1.83	1.60	1.10	1.87	1.75	1.43	1.76	1.47

Values represent the mean±SE (n=3).

RG, red ginseng.

¹⁾No. of times being steamed.

²⁾Ginsenosides Rg₂+Rg₃+Rg₅+Rg₆+Rh₁+Rh₄+Rk₁+Rk₃+F₁+F₄.

³⁾Sum total of individual ginsenoside contents.

Rg₆, Rh₁, Rh₄, Rk₁, Rk₃, F₁, F₄), main components of black ginseng, which peaked in ginsengs steamed 8 times (1.15%), followed by those steamed 9 times (1.05%), 6 times (0.98%), 5 times (0.96%), 7 times (0.94%), 4 times (0.86%), 3 times (0.75%), twice (0.71%) and once (0.43%), showed that those steamed 8 times contain about 2.7 times as much prosapogenin as those steamed only once as shown in Table 2.

At the same time, the contents of ginsenoside Rk₁ and Rg₅ peaked in red ginsengs steamed 4 times (0.44%), followed by those steamed 8 times (0.35%), 9 times (0.35%), 7 times (0.30%), 6 times (0.24%), 5 times (0.17%), 3 times (0.75%), twice (0.76%) and those steamed once (0.43%), showing that those steamed 4 times contained about 16 times as much of them as those steamed only once.

On the other hand, when it comes to the contents of

ginsenoside Rg₃, a physiologically activating substance [10-12] such as anticancer activities, red ginsengs steamed 8 times amounted to 0.28%, topping the list, followed by those steamed 9 times (0.27%), 7 times (0.20%), 6 times (0.17%), 4 times (0.14%), 5 times (0.14%), 3 times (0.11%), twice (0.05%) and those steamed only once (0.02%).

A comparison and analysis of the total content of prosapogenin components in the process of steaming ginsengs starting from once to 9 times confirmed that the contents of prosapogenin peaked in red ginsengs steamed 8 times. Accordingly, it is thought that steaming ginsengs 8 times rather than 9 times (black ginsengs) would be the optimal steaming condition for the development of red ginseng products containing high concentration and high function prosapogenin.

Thus, high function ginsengs containing prosapogenin

substances (ginsenoside Rg₂, Rg₃, Rg₅, Rg₆, Rh₁, Rh₄, Rk₁, Rk₃, F₁, and F₄) can be produced by adjusting ginseng steaming conditions, expectedly making it possible to develop world-renowned red ginseng products.

ACKNOWLEDGEMENTS

This research was supported by Technology Development Program for Food, Ministry for Food, Agriculture, Forestry and Fisheries, Korea (2010).

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