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원적외선 및 증숙 처리에 따른 인삼 잎의 Protopanaxadiol Ginsenosides 변화

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Changes of Protopanaxadiol Ginsenosides in Ginseng Leaves by Far Infrared and Steaming Heat Treatments

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ABSTRACT : PPD ginsenosides in ginseng leaf were analyzed to determine effects of either FIR heat or steaming heat treatment. Among the PPD ginsenosides, Rb1, Rc and Rb3 forming four glycoside-attached aglycons were increased as FIR heat temperatures were increased from 60 to 120 ℃, while Rb3 was decreased. In addition, FIR heat treatment was effective to increase Rd forming a three glycoside-attached aglycon. Rg3 and Rh2 were not increased by the FIR heat treatment. In steaming heat treatment, Rb1 was significantly decreased, while Rb2 was increased. Rd was also increased by increased steaming temperature, yet its content was lower than in the FIR heat treatment. However, the steaming heat treatment increased yields of Rg3 and Rh2, which were not observed in the FIR heat treatment. Thus, FIR heat treatment was beneficial to efficient products of Rb1, Rc, Rb3 and Rd. Steaming heat treatment was effective to higher collection of Rb2, Rg3 and Rh2.

Key Words **:** Far Infrared Ray, Ginseng Leaf, Protopanaxadiol Ginsenosides, Steaming

INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer) has been popularly cultivated in Korea with the awareness of the most profitable medicinal crop. In resent several decades, the yield of ginseng production has been getting increased by the development of cultivating techniques, such as fertilizer use and pesticide application (Jo & Won, 1995). In commercial aspect, ginseng root is the main trade part among ginseng tissues, with exception to seed sale for next cultivation. Ginseng leaf has commercially thought to waste part and discarded when ginseng roots were harvested. Recently, numerous researches reported that ginseng leaf contained various ginsenosides (Li *et al*., 1996; Wang *et al*., 2006). The leaf ginsenosides were compared with root ginsenosides in order to potential use of purified ginsenosides from the leaves (Popovich & Kitts, 2004). The wasted leaves may be potentially used if certain ginsenosides from the leaves were purified and determined to profitable products in the purpose of pharmaceutical use. However, it is true that ginsenoside analysis of ginseng leaf by the effect of heat processes were still less investigated, while the analysis of its root have been numerously studied.

Ginsenosides are composed of triterpene glycosides and classified to dammarane triterpene types with either protopanaxadiol (PPD) or protopanaxatriol (PPT) aglycon moieties and oleanolic acid type (Tanaka & Kasai, 1984). Ginsenosides forming aglycon with glycosides were

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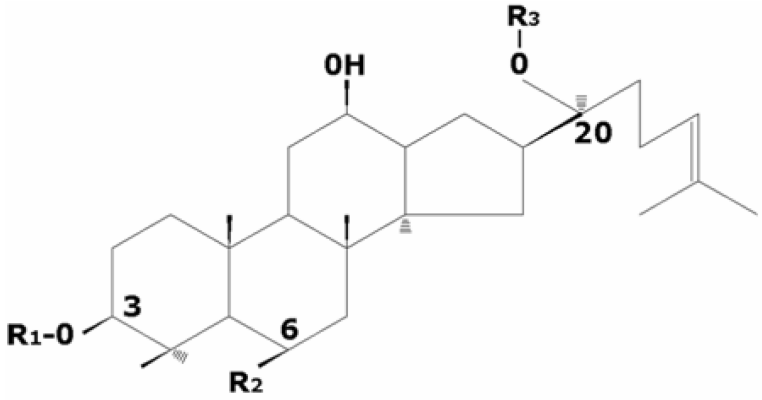

PPD ginsenosides	R1	R2	R3
Rb1	glc-glc	H	glc-glc
Rc	glc-glc	H	glc-ara (f)
Rb2	glc-glc	H	glc-ara (p)
Rb3	glc-glc	H	glc-xyl
Rd	glc-glc	H	glc
Rg3	glc-glc	H	H
Rh2	glc	H	H

Fig. 1. Structure of PPD ginsenosides evaluated in this experiment.

transformed to other ginsenosides by detachment of glycosides. It is well known that the transformations were occurred by heat, acidic hydrolysis and certain enzyme activity (Kim *et al.*, 2007; Han *et al.*, 1982; Cheng *et al.*, 2006). Of the three types of ginsenosides, PPD ginsenosides are Rb1, Rb2, Rb3, Rg3, Rd, Rc, Rh2, F_2 and Compound K. We analyzed contents of certain PPD aglycon moieties (Fig. 1), including Rb1, Rb2, Rb3, Rg3, Rd, Rc, and Rh2, in Korean ginseng leaves treated to two types of heat processes in order to which process is effective to high yield of certain PPD ginsenosides.

MATERIAlS AND METHODS

1. Materials

Leaves aged 5 year-old ginsengs were collected from a ginseng cultivation area, Hengseong, Korea, in mid-September 2007. Fresh green leaves were selectively harvested. Standards of PPD ginsenosides for HPLC analysis were purchased from several chemical companies.

2. Heat treatments to ginseng leaves and extraction

The collected leaves were quickly rinsed using distilled water. After removing moistened droplets, the samples were put into either steaming by an autoclave or drying by a far infrared ray chamber. In our preliminary test, time of heat treatment by FIR was optimized to 10 min when dry weight of ginseng leaves were maintained to 10% of the fresh weight in 60℃ treatment. Thus, 10 min of FIR treated leaves under 60, 90 and 120℃, respectively. In the case of steaming treatments for 60, 90 and 120℃, real steaming time in autoclave was 10 min during overall 30 min running of autoclave when the overall time was included time for initial temperature increase, time for experimental temperature and time for final temperature decrease. After steaming, the leaves were dried in a 60℃ dry oven for 2 h.

Dried leaves were finely ground by a turbo mill (Korea Energy Co., Hong cheon, Korea). Leaf powder (2 g) per sample was suspended in 100 mL of methanol for 24 h for extraction. Supernatant was taken after filtration using #1

filter paper (Whatman, Florham Park, NJ, USA) and residue part was extracted again with the same condition of the first extraction). After pooling the supernatants, the solution part was removed using a vacuum rotary evaporator (N-1000, Eyela, Tokyo, Japan) set in a 38℃ water bath. The pellets of samples were collected and lyophilized. The lyophilized samples were reserved in a −80℃ freezer until analysis.

3. PPD ginsenoside analysis by HPLC

Each extracted sample was dissolved in 100 mL of d · dH_2O on a fraction funnel. Lipophillic components of the leaf extracts were removed by a solvent fractionation using ethyl acetate. Ginsenosides were separated by n-butanol fractionation. Butanol was removed using a vacuum rotary evaporator set in a 38℃ water bath. After lyophilization, ginsenosides in the pellets were analyzed with HPLC system (CBM-20A, Shimadzu Ltd., Kyoto, Japan) with 2 gradient pump systems (LC-20AT, Shimadzu), a UV-detector (SPD-10A, Shimadzu). A column used was Gemmini C18 column (㎛, 100 × 4.6 ㎜, Phenomenex. Inc., Torrance, CA, USA). The flow rate of the mobile phase solution was controlled to 1.0 mL/min. The mobile phase was programmed as following set: solution A (0.4%, v/v, formic acid in d · dH_2O) and solution B (0.4%, v/v, formic acid in acetonitrile), with a gradient elution programmed as follows: 28-32% of solution B for 0 to 5 min, 32-37% of solution B for 5 to 15 min, 37-40% of solution B for 15 to 20 min, and 40-70% of solution B for 20 to 40 min. Sample injection volume was 10 μL (10 ㎎/mL), and peaks were monitored at 203 ㎚. The HPLC analysis was conducted on the basis of a chromatogram profile of standard PPD ginsenoside (Fig. 2).

RESULTS AND DISCUSSION

1. PPD Ginsenoside contents

It has been well known that ginsenosides transformed from four glycoside ginsenoside types to less glycoside-attached ginsenoside types by three factors, including heat, acidic hydrolysis and enzyme activity (Kim *et al*., 2007; Han *et al*., 1982; Cheng *et al*., 2006). Among the four glycoside PPD ginsenoside types, Rb1, Rc, Rb2 and Rb3 were analyzed in this heat treating experiment. Also, a three-glycoside ginsenoside type, Rd, was measured, which was transformed from the four glycoside-attached ginsenosides. In addition, a two glycoside-attached ginsenoside (Rg3) and a glycoside-attached Ginsenoside (Rh2) were measured. Fig. 3 shows that PPD ginsenosides biodegradation processes were occurred by detaching glycosides from its aglycon. In the leaf of Korean ginseng, Rb1 (2.54 ㎎/g dry weight) was the highest content among the PPD ginsenosides in the ambient water extraction (Table 1). Contents of Rc, Rd and Rb3 were the secondly high amount groups, exhibiting 1.28, 0.90 and 0.88 ㎎ per gram dry weight, respectively, whereas Rg3 (0.06 ㎎/g dry weight) and Rh2 (0.01 ㎎/g dry weight) were the lowest content group. However, ginsenoside contents in ginseng leaf were likely to differ by ginseng species and growth stages (Shi *et al*., 2007). In addition, it is true that contents of

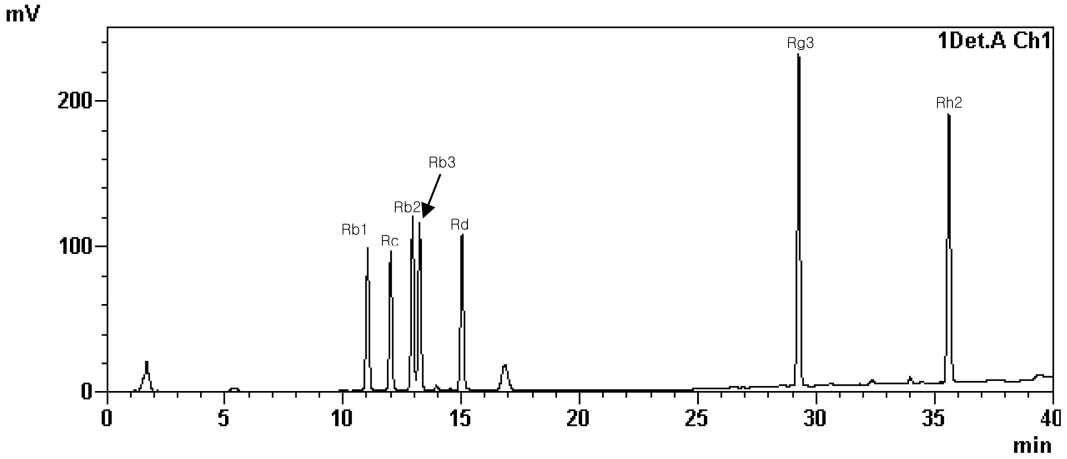

Fig. 2. Chromatogram of standard PPD ginsenosides in HPLC.

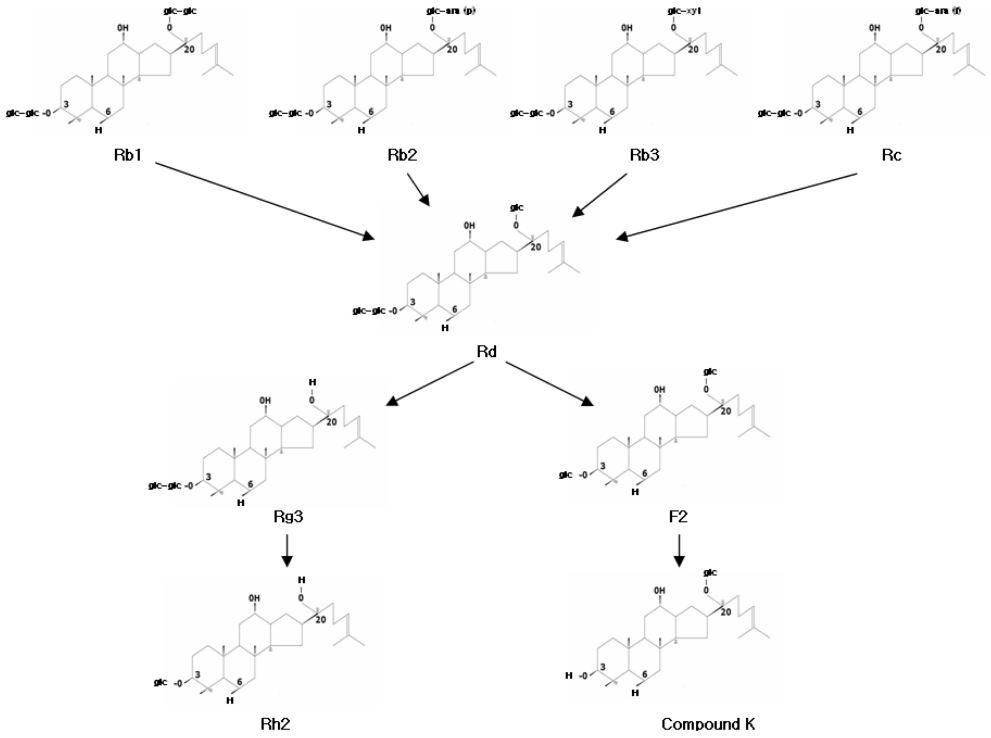

Fig. 3. Degradation processes of PPD ginsenosides.

Table 1. PPD ginsenoside contents in ginseng leaves by heat treatments.

	Ginsenoside content (㎎/g leaf dry weight)			
	Far infrared ray treatment (℃)			
	Control	60	90	120
Rb1	2.54 b*	2.35 b	2.94 a	3.01 a
Rc	1.28 d	1.46 c	2.44 b	2.61 a
Rb2	0.48 c	1.03 b	2.39 a	2.36 a
Rb3	0.88 a	0.43 b	0.61 b	0.56 b
Rd	0.90 c	1.62 b	3.67 a	3.86 a
Rg3	0.06 a	0.04 a	ND†	0.06 a
Rh2	0.01 a	0.02 a	ND	0.02 a
	Steaming by autoclave (℃)			
	Control	60	90	120
Rb1	2.54 a	0.98 bc	1.30 b	0.70 c
Rc	1.28 a	0.99 a	1.29 a	1.14 a
Rb2	0.48 c	0.74 bc	0.81 b	3.05 a
Rb3	0.88 ab	0.72 b	1.21 a	0.96 ab
Rd	0.90 c	1.42 b	1.82 a	1.57 ab
Rg3	0.06 b	0.08 b	0.14 a	0.13 a
Rh2	0.01 b	0.02 ab	0.02 ab	0.03 a

*Values in the same row followed by different letters are significantly different at the 5% level based on Tukey's Student Range Test.
†Represents 'not detected'.

ginsenosides are highly variable in dependent of ginseng extraction procedures. It was reported that leaf of American ginseng (*Panax quinquefolius* L.) contains Rb1 as the highest content among PPD ginsenosides, where hot water or ethanol

extractions were accomplished (Popovich & Kitt, 2004).

2. PPD ginsenoside contents by heat treatments

Several studies have reported that bioactive components were increased by FIR and steaming heat treatments (Eom *et al*., 2007; Eom *et al*., 2008; Kim *et al*., 2007). Table 1 shows changes of PPD ginsenoside contents by either FIR heat treatment or steaming heat treatment. Although temperature was equally applied to ginseng leaves in the both treatments, certain PPD ginsenoside contents were differed. In the FIR treatment, Rb1, Rc, and Rb2 were significantly increased where FIR temperatures were increased from 60 to 120℃, while Rb3 was significantly decreased in FIR treatment as compared with non FIR treatment control. Rd was increased when temperature in the FIR treatment. Rg3 and Rh2 were not significantly differed between FIR and non FIR treatments. Otherwise, steaming heat treatments with ginseng leaf significantly decreased Rb1, yet less affected to Rc and Rb3. In the case of Rb2, both treatments increased Rb2. It is indicated that an efficient Rb2 extraction from ginseng leaves should be demanded certain amount of heat process. Although the steaming also increased Rd content, it was less increased as compared with FIR heat. Rg3 and Rh2 were increased as temperature was increased.

CONCLUSION

Patterns of PPD ginsenoside contents in ginseng leaves were distinctly differed by application of heat treatments, such as FIR heat dry and steaming treatments. FIR heat treatment was beneficial to efficient product of Rb1, Rc, Rb3 and Rd. Steaming treatment was effective to higher collection of Rb2, Rg3 and Rh2.

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