Korean J. Ginseng Sci.
Vol. 10, No. 2 (1986)

Effect on Stability of Ginseng Saponins by Various Physical and Chemical Treatments

Jae-Ho Do, Jin-Gyu Jang, Kwang-Seung Lee and Hyun-Soon Sung
Korea Ginseng & Tobacco Research Institute
(Received Oct. 15, 1986)

물리화학적 처리가 인삼 Saponin의 안정성에 미치는 영향

도재호 · 장진규 · 이광승 · 성현순
한국인삼연초연구소
(1986년10월15일 접수)

Abstract

To investigate the stability of ginseng saponin, various physical and chemical treatments for red ginseng alcohol extract (70% ethyl alcohol) were carried out, and then the variations of ginseng saponin in extract were investigated by high performance liquid chromatography (HPLC) method.

Irradiation of γ-ray, and ultraviolet ray, sonocatalysis by ultrasonicator, treatment of electronic range, catalytic ozonation did not or slightly affect degradation of ginseng saponins, but they were degraded by heat treatment.

Introduction

Korean ginseng (*Panax ginseng* C.A. Meyer) has many biological activities such as increasing stamina, having antipyretic, neurotonic, diuretic, antihemorrhagic, cardiotonic effects, preventing tuberculosis, controling blood pressure, normalizing the gastrointestinal system, strengthening liver functions, treating tumors and diabetes, etc [1]. Korea ginseng contains many kinds of saponins, these saponins also exhibit numerous biological and pharmacological effects. Up to the present, a total of 17 saponins have been identified; ginsenoside Ro, Ra_1, Ra_2, Ra_3, Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, Rh_2 and 20-gluco-Rf. Among various saponins, ginsenoside Rb_1 exhibits inhibitory effects on the central nervous system, analgesic and antipyretic effects, promotes synthesis of protein, nucleic acid and choresterol, and inhibits neutral

lipid breakdonw, ginsenoside Rb_2 exhibits inhibitory effects on the central nervous system, promotes synthesis of DNA, RNA and protein. Ginsenoside Rc exhibits inhibitory effects on the central nervous system, promotes DNA, RNA and serum protein synthesis, and ginsenoside Re exhibits inhibitory effects on the central nervous system, promotes DNA and RNA synthesis. Ginsenoside Rg_1 stimulates the central nervous system, promotes recovery from fatigue, and DNA, RNA synthesis, ginsenoside Rg_2 provents platelet aggregation. Ginsenoside Rh_2 inhibits the proliferation of cancer cells, ginsenoside Ro exhibits anti-inflammatory effects, has detoxifying action and antithrombotic action, and also ginsenoside Ro, Rb_1, Rb_2, Rc, Re, Rg_1, Rg_2 stimulate plasmin activity[2]. As above mentioned, ginseng saponins contained in ginseng are main compounds which exhibit the biological and pharmacological effects. These ginseng saponin could be degrated during manufacturing various ginseng products. Yang *et al.*[3] and Shoji[4] reported studies on effects of pH and heat treatment on the stability, and hydrolysis by dilute-hydrochrolide solution of ginseng saponin. In this experiments, in order to protect the disintergration of ginseng saponin during manufacturing of various red ginseng products, after red ginseng alcohol extract were treated with various physical and chemical treatments, we investigated by HPLC whether ginseng saponins in extract were degraded or not degraded by treatments.

Materials and Methods

Red ginseng alcohol extract Red ginseng alcohol extract (extract manufactured by extraction and concentration with 70% ethyl alcohol) manufactured by Office of Monopoly in 1986 was used in this experiment[5].

Physical and chemical treatment γ-ray irradiation of red ginseng alcohol extract was carried out with ^{60}Co-γ-ray irradiation instrument in Korea Advanced Energy Research Institute, ultra-violet ray irradiation was carried out with ultra-violet lamp (15W, 2537Å) for sterilization.

Sonocatalysis was done with ultrasonicator(Brannsonic 1510 U.S.A., 50W, 20KHz), and ozonation was performed with ozone generator(1.5g/hr). Electronic range treatment was performed with Mitsubishi electronic range(580W, 2450MHz) and heat treatment was carried out with autoclave.

Analysis of ginseng saponin Saponins in red ginseng alcohol extract treated with various physical and chemical treatment were extrcted by method of Namba and Shibata[6,7] and each ginsenoside was analyzed by high performance liquid chromatograph, and the conditions of HPLC for analysis of ginseng saponins were shown in Table 1.

Table 1. Conditions of HPLC for analysis of saponins in red ginseng alcohol extract

Model	;	Waters Associate Model 244
Column	;	μ-Bondapak carbohydrate analysis (4mm × 30cm)
Solvent	;	Acetonitril:H_2O:BuOH (80:20:15)
Detector	;	Differential Refractometer (RI)
Sensitivity	;	8X
Flow rate	;	2.0 ml/min
Chart speed	;	1 cm/min
Injection vol.	;	10 μl

Results and Discussion

Effect of γ-ray irradiation To investigate the effect on stability of ginseng saponin by γ-ray irradiation, red ginseng alcohol extract was irradiated by ^{60}Co-γ-ray at a range of 0-50KGy (1KGy = 100Krad) and the results were summarized in Table 2. As shown

Table 2. Effect of γ-ray irradiation on stability of ginseng saponin

Irradiation dosage(KGy)*	Ginsenosides (Unit: μg/10μl)					
	Rg_1	Re	Rd	Rc	Rb_2	Rb_1
0	47.8	68.6	49.0	111.1	130.3	227.1
10	48.3	70.4	48.6	113.7	124.9	225.1
15	47.5	68.3	50.8	112.9	118.7	213.2
50	48.0	70.0	49.2	112.4	115.1	208.9

* 1 KGy is equal to 100 Krad.

in Table 2, even irradiation dossage of 50 KGy did not affect the degradation of ginseng saponin. When water molecule is irratiated by γ-ray, various free radicals are formed by ionization[8].

$$H_2O + \text{Radiation} \rightarrow H_2O^+ + e^-$$
$$e^- + H_2O \rightarrow H_2O^-$$
$$H_2O \rightarrow H^+ + OH^-, \quad H^+ + e^- \rightarrow H$$
$$H_2O^+ \rightarrow H^+ + \dot{OH}, \quad H_2O^- \rightarrow H + OH^-$$
$$OH^- \rightarrow \dot{OH} + e^-$$
$$H + O_2 \rightarrow \dot{HO}_2, \quad 2\dot{HO}_2 \rightarrow O_2 + H_2O_2$$

Here, H formed from water molecule by the reaction of radient ray is very powerful reductant, and $\dot{OH}$, $\dot{HO}_2$ and H_2O_2 is powerful oxidant. By the radiation, contents of triol group saponins in red ginseng extract did not change, but contents of diol group

saponins slightly decrease. It means that triol group saponins such as ginsenoside Rg_1, Re and Rd are very stable for radiation. In susceptibility of vitamins against for ionizing radiation, ascorbic acid, thiamin, pyridoxine, cyanocobalamin, β-carotene, vitamin A, E, K and nicotinic acid are sensitive to radient ray, but riboflavin, panthothenic acid, biotin, folic acid, choline and vitamin D are stable. And the senstive vitamins for radient ray are apt to degrade by one factor of heat treatment, oxygen and light[9].

Effect of ultra-violet ray irradiation In order to investigate the effect on stability of ginseng saponin by the irradiation of ultra-violet ray, red ginseng alcohol extract (13° Bx) was irradiated by ultra-violet lamp for 0-50 hrs, and the results are shown in Table 3. As shown in Table 3, each ginsenoside was very stable for the irradiation of ultra-violet ray. The energy of ultra-violet ray is less than that of ionizing radiation, so its sterilizing power and transmittance are also feeble. And because ultra-violet ray is preterentially absorbed to nucleic acids, it changes the structure of DNA chain and forms thymin dimers in intracellular of microorganisms[10].

Table 3. Effect of UV-ray irradiation on stability of ginseng saponin

Irradiation time (hrs)	Ginsenosides (Unit: μg/10μl)					
	Rg_1	Re	Rd	Rc	Rb_2	Rb_1
0	47.8	68.6	49.0	111.1	130.3	227.1
5	48.2	68.2	48.6	111.7	130.9	227.9
15	47.4	69.1	49.2	110.8	129.5	226.4
30	48.6	67.7	49.5	111.5	131.2	227.3
50	47.4	67.4	49.8	112.5	131.8	227.7

Effect of ultrasonic treatment Cell disintergration by ultrasound is caused by cavitational forces producing shock waves, chemical attack by free radicals, or cavitational microstreaming. Whatever the mechanism of disintergation, the ultrasonic treatment continues to be an effective means for rupturing bacterial cells[11]. During ultrasonication, foaming can cause protein dematuration, and the cavitation phenomenon promotes oxidation of oxygen-sensitive enzymes and unsaturated lipids[12]. There are no problems for the disintergration of large molecules, because red ginseng was heat-treated at 100°C for 2-3 hrs during manufacturing red ginseng. After sonication for red ginseng alcohol extract, the variations of ginsenoside contents in extracts were investigated, and the results are shown in Table 4. Ultrasonic treatment for 10 min did not affect the variation of ginsenoside content or disintergration of ginseng saponin.

Effect of ozonation In order to investigate effect on ginseng saponin by ozonation, ozone bubblings for red ginseng alcohol extract were carried out with ozone generator for 0-20 min. Ozonation also did not affect the contents of triol group saponins,

Table 4. Effect of ultrasonic treatment on stability of ginseng saponin

Time (min)	Ginsenosides (Unit: μg/10μl)					
	Rg_1	Re	Rd	Rc	Rb_2	Rb_1
0	47.8	68.6	49.0	111.1	130.3	227.1
2	47.0	68.0	47.4	109.3	131.2	226.0
5	46.2	67.6	49.3	112.3	132.6	228.4
10	47.7	69.2	50.7	112.6	131.8	229.5

Table 5. Effect of ozonation on stability of ginseng saponin

Time (min)	Ginsenosides (Unit: μg/10μl)					
	Rg_1	Re	Rd	Rc	Rb_2	Rb_1
0	47.8	68.6	49.0	111.1	130.3	227.1
5	45.2	64.8	49.8	111.4	129.9	228.9
10	48.0	66.3	47.6	101.6	116.2	201.0
15	46.4	63.9	47.8	102.7	105.3	183.4
20	47.1	64.7	46.5	74.7	73.4	150.9

but diol group saponins in red ginseng alcohol extract were slightly disintergrated (Table 5).

Effect of electronic range Red ginseng alcohol extract was treated by electronic range, cooking utensil, at time intervals, and then contents of ginseng saponin in extract was investigated. As shown in Table 6, contents of all ginsenosides were not changed. In case of electromagnetic wave spectra, radio waves, radar waves (10^7 - 10^9Hz) and infrared rays(10^{12} - 10^{14}Hz), radient of low frequency, affect molecules by heat vibration of low proton energy, and then generally heat generated by heat vibration has sterilization reaction[13]. But ginseng saponins(diol and triol group) were not affected by treatment of microwave, it means that ginseng saponin is very stable in its structure.

Table 6. Effect of electronic range treatment on stability of ginseng saponin

Time (sec)	Ginsenosides (Unit: μg/10μl)					
	Rg_1	Re	Rd	Rc	Rb_2	Rb_1
0	47.8	68.6	49.0	111.1	130.3	227.1
10	47.9	69.2	46.8	112.5	131.3	227.1
20	46.9	67.4	47.1	113.0	133.1	225.3
40	44.6	64.9	46.3	109.6	133.1	226.2
60	46.9	64.8	46.0	110.3	132.5	224.8
90	48.5	64.9	46.5	109.7	131.4	220.5

Effect of heat treatment Heat treatments cause the structural change of organic substances such as proteins, nucleic acids, enzymes etc, and this reaction was almost irreversible. Red ginseng alcohol extract was heat-treated for 1 hr at 100, 110 and 120°C, and then the contents of ginsenosides were investigated. As shown in Table 7, content of each ginsenoside was not changed by heat treatment at 100°C for 1 hr, but by treatment at 110 and 120°C, the contents of ginsenoside Re, Rc, Rb_2 and Rb_1 was decreased with an increase of temperature except contents of ginsenoside Rg_1 and Rd. Ginsenoside Rd was heat-stable substance, because its content was no changed by heat treatment of 120°C. But content of ginsenoside Rg_1 was increased with an increase of temperature. We suppose that the increase of content of ginsenoside Rg_1 was caused by transforming ginsenoside Re and 20-gluco ginsenoside Rf into ginsenoside Rg_1 by heat degradation. In a structure of ginseng saponin, that is, if glucose-rhamnose or glucose-glucose in C-6 position of sugar moiety of ginsenoside Re or 20-gluco ginsenoside Rf was cloven by chemical and physical treatment, both ginsenoside Re and 20-gluco ginsenoside Rf can be transformed into ginsenoside Rg_1 [14]. From above results, we knew that ginseng saponin is very stable substance in structure, and that diol group saponin is more unstable substance than triol group saponin.

Table 7. Effect of heat treatment on stability of ginseng saponin

Temperature (°C)	Ginsenosides (Unit: μg/10μl)					
	Rg_1	Re	Rd	Rc	Rb_2	Rb_1
No treatment	47.8	68.6	49.0	111.1	130.3	227.1
100	49.4	63.1	47.8	111.9	131.0	212.3
110	56.1	57.6	46.3	108.3	123.9	193.7
120	79.3	37.8	42.7	88.3	104.8	155.6

*Heat treatment of ginseng extract was carried out for 1 hr at given temperature.

요 약

인삼 엑기스 제품을 제조·가공할 때 여러가지 물리화학적 처리방법이 인삼의 주요성분인 사포닌의 안정유지에 미치는 영향을 조사하였다. 자외선 조사·전자렌지 및 초음파 처리에 의해서는 거의 모든 인삼의 사포닌이 분해되지 않았으나 ozone bubbling에 의해서는 ginsenoside Rc, Rb_2 및 Rb_1이 상당히 분해되었고 감마선을 50 KGy(5Mrad)조사 하였을 때 ginsenoside Rg_1, Re, Rd의 함량은 변하지 않았으나 Rc, Rb_2, Rb_1은 조사선량이 증가됨에 따라 약간씩 분해가 일어났다. 특히 열처리의 경우는 온도가 높을수록 거의 모든 사포닌에서 분해가 많이 일어났으나 ginsenoside Rg_1은 오히려 그 양이 증가하는 경향을 보였으며 이는 사포닌 구조상으로 볼 때 ginsenoside Re와 20—gluco

ginsenoside Rf의 glucose-rhamnose, glucose-glucose가 탈리되어 ginsenoside Rg_1으로 전환된 것으로 추정된다.

Literature Cited

1. Korea Ginseng & Tobacco Research Institute : An introduction to Korean ginseng, p. 23, 2nd edition (1985).
2. Korea Ginseng & Tobacco Research Institute : An introduction to Korean ginseng, p. 10, 2nd edition (1985).
3. Yang, J. W., J. H. Do, H. S. Sung and S. K. Hong : *Korean J. Ginseng Sci.* **6,** 25 (1982).
4. Shoji, J.:化學の 領域 **35,** 9(1981)
5. 전매청 : 홍삼 및 홍삼제품품질교범(1982).
6. Namba, T., M. Yoshizahi, T. Tomomori, K. Kobashi, K. Kitsui and J. Hase : *Yakugaku Zasshi* **94,** 252(1974).
7. Shibata, S., O. Tanaka, T. Ando, M. Sado, S. Tsushima and T. Ohsawa : *Chem. Pharm. Bull.* **14,** 595 (1966).
8. 芝崎 勳 : 新・食品殺菌工學, 光琳社 p.307 (1983).
9. 芝崎 勳 : 新・食品殺菌工學, 光琳社 p.325 (1983).
10. 芝崎 勳 : 新・食品殺菌工學, 光琳社 p.344 (1983).
11. Dobrogosz, W. J. : Metabolism in Manual of Methods for General Bacteriology, p. 369, edited by P. Gerhardt, American Society for Microbiology, Washington, DC (1981).
12. Schnaitman, D. A. : Cell fractionation in Manual of Methods for General Bacteriology, p. 54, edited by P. Gerhardt, American Society for Microbiology, Washington, DC (1981).
13. 芝崎 勳 : 新・食品殺菌工學, 光琳社 p.293 (1983).
14. Korea Ginseng & Tobacco Research Institute : An introduction to Korean ginseng, p. 116, 2nd edition (1985).