

## A New Processed Ginseng with Fortified Activity

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### ABSTRACT

A new processed ginseng with fortified activity is developed. The process comprise with the heat treatment of fresh or white ginseng at higher temperature and pressure than those used for the preparation of red ginseng. This new processed ginseng showed 7 times higher antioxidant activity and more than 30 times stronger vasodilating activity than those shown in raw ginseng. Other activities found in the new processed ginseng include cancer chemoprevention, antinephrotoxic, and antineurotoxic activities. Less polar ginsenosides isolated from processed ginseng exhibited anti-platelet aggregation activity and anti-cancer activity. Many ginsenosides were isolated from this new processed ginseng, namely 20(S)-Rg<sub>3</sub>, 20(R)-Rg<sub>3</sub>, Rg<sub>5</sub>, Rg<sub>6</sub>, F<sub>4</sub>, Rh<sub>4</sub>, 20(S)-Rs<sub>3</sub>, 20(R)-Rs<sub>3</sub> and Rh<sub>4</sub>. In addition to these known compounds, seven new ginsenosides, named as ginsenoside Rk<sub>1</sub>, Rk<sub>2</sub>, Rk<sub>3</sub>, Rs<sub>4</sub>, Rs<sub>5</sub>, Rs<sub>6</sub>, and Rs<sub>7</sub> were isolated. The major constituents of new processed ginseng were 20(S)-Rg<sub>3</sub>, 20(R)-Rg<sub>3</sub>, Rk<sub>1</sub> and Rg<sub>5</sub> which are minors in red ginseng. Since the chemical constituents and biological activities of this new processed ginseng are quite different from those of white or red ginseng, we designated it as "*sun ginseng* (仙蔘)."

### Introduction

Obviously, herbal drug contains many kinds of chemical constituents, some of which are biologically active, while others are inactive or toxic. In past, science was not developed enough to remove inactive or toxic components, or to purify the active constituent, consequently patients have to take not only active principles but also inactive or toxic components.

Traditionally many kinds of herbal drugs have been used as medicine after process. Chemical constituents in the herbal drug might be modified by the process, which results change in activity or toxicity. The aim of process of herbal drug might be to increase biological activity or to decrease the side effect. Most frequently used processing method in oriental medicine are roasting and steaming. Many of chemical constituents would be modified during the process, which may cause the change in biological activity. Therefore, the compound which is modified through the process is closely related to the biological activity of herbal drug. However, the chemical and biological change during the process of herbal drug are not well known.

Ginseng is one of herbal drug which is used after processing[1]. Generally ginseng is used in the form of a white ginseng which is prepared by drying the fresh ginseng at a room temperature or of a red ginseng which is processed by steaming the fresh ginseng at a temperature of 98 to 100 °C.

Recently, many scientific studies on ginseng have been revealed biological activities of ginseng. Some reported activities are prevention of aging, anti-arteriosclerosis, treatment of hyperlipidemia, treatment of hepatic insufficiency, protection from radiation injury, anti-thrombic, anti-stress, anti-diabetic, anti-tumor, anti-hypertensive effects, etc.

It is interesting that the red ginseng shows stronger activity than white ginseng in most cases, and the red ginseng has been recognized as a valuable medicine[2-4]. We can easily assume that the differences in biological activity of white ginseng and red ginseng come from the difference in their chemical constituents. Recently many minor compounds are reported from red ginseng. Ginsenoside Rg<sub>3</sub>, Rg<sub>5</sub>, Rg<sub>6</sub>, Rh<sub>2</sub>, Rh<sub>3</sub>, Rh<sub>4</sub> and Rs<sub>3</sub> are some examples[5-13]. These compounds are absent or, if any, present in a trace amount in white ginseng.

In this study we investigated on a new processed ginseng which is prepared by heat treatment of raw ginseng at a higher temperature than the temperature used for the preparation of red ginseng. As a result, we found that the trace components of a red ginseng are significantly increased as well as novel components are produced in a new processed ginseng, and some biological activities are greatly enhanced.

## Materials and Methods

### *Isolation of ginsenosides from processed ginseng*

Processed ginseng was prepared by heat treatment of the white ginseng (30kg) at 120 °C for 3 hours. The methanol extract of processed ginseng was partitioned between water and CH<sub>2</sub>Cl<sub>2</sub>. The aqueous phase was partitioned again between water and *n*-BuOH. The *n*-BuOH phase was taken and evaporated under vacuum to yield the *n*-BuOH fraction. The *n*-BuOH fraction was chromatographed on a silica gel column eluting with EtOAc : MeOH : H<sub>2</sub>O (40:1:1 → 10:1:1) to afford 5 fractions. The ginsenosides were isolated by repeated silica gel column chromatography and/or reverse phase HPLC and/or AgNO<sub>3</sub>-impregnated prep-TLC[14]. The structure of ginsenosides was determined by various spectroscopic methods.

### *Analysis of ginsenosides by HPLC/ELSD*

The processed ginseng was refluxed with methanol for 3h. Solvent was evaporated *in vacuo* and the residue was partitioned between diethyl ether and water. The water layer was further extracted with water-saturated *n*-butanol. The *n*-butanol fraction was dried *in vacuo* and the residue was dis-

solved in methanol, which was subjected to HPLC analysis[15].

#### *Vasodilation activity of processed ginseng*

Male Sprague-Dawley rats were sacrificed and their thoracic aortae were removed and placed in a modified Krebs-Ringer bicarbonate solution. The aortic rings were suspended horizontally between two stainless steel stirrups in organ chambers filled with 25ml of control solution(37°C, pH 7.4) and bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

One of the stirrups was anchored to the organ chamber and one was connected to a transducer coupler (Narco bio-system) for the recording of isometric tension. The aortic rings were stretched progressively to the optimal tension (2g) before the addition of phenylephrine(10<sup>-6</sup>M). Once the plateau of the contraction to phenylephrine was obtained, the aortic rings were rinsed three times with warm (37°C) control solution. After a resting period (30min), the aortic rings were exposed again to phenylephrine(10<sup>-6</sup>M). When the contraction had stabilized, acetylcholine(10<sup>-6</sup>M) was added to test the presence or the absence of the endothelium. The organ chambers were rinsed three times with warm(37°C) control solution before the addition of indomethacin(10<sup>-5</sup>M) to prevent the production of endogenous vasoactive prostanoids. A cumulative concentration-response curves to ginseng extracts were obtained following the contraction of aortic rings with phenylephrine(10<sup>-6</sup>M)

## **Results and Discussion**

#### *Chemical constituents of processed ginseng*

Since the major constituents of ginseng is ginsenosides, we focused our first effort to the separation and identification of ginsenosides in a processed ginseng. Generally the content of less polar ginsenosides was greatly increased in the processed ginseng. Many saponins were purified and identified from processed ginseng. Previously reported ginsenosides as a minor constituents of red ginseng were isolated, which includes 20(S)-Rg<sub>3</sub>, 20(R)-Rg<sub>3</sub>, Rg<sub>5</sub>, Rg<sub>6</sub>, F<sub>4</sub>, Rh<sub>1</sub>, Rh<sub>2</sub>, Rh<sub>4</sub>, 20(S)-Rs<sub>3</sub> and 20(R)-Rs<sub>3</sub>. In addition to these known ginsenosides, seven new ginsenosides were isolated, which were named as ginsenoside Rk<sub>1</sub>, Rk<sub>2</sub>, Rk<sub>3</sub>, Rs<sub>4</sub>, Rs<sub>5</sub>, Rs<sub>6</sub> and Rs<sub>7</sub>.

Ginsenoside Rk<sub>1</sub> was a dehydrated compound of ginsenoside Rg<sub>3</sub> at C-20 position. Unlike ginsenoside Rg<sub>5</sub>, new double bond formed by dehydration was produced between C-20 and C-21 carbons. Ginsenoside Rk<sub>2</sub> and Rk<sub>3</sub> were the mono-deglycosylated products of ginsenoside Rk<sub>1</sub> and Rg<sub>6</sub>, respectively.

Ginsenoside Rs<sub>5</sub> was the mono-O-acetylated compound of Rk<sub>1</sub> at 6''-O position. Ginsenoside Rs<sub>6</sub> and Rs<sub>7</sub> were the mono-O-acetylated compound of Rh<sub>4</sub> and Rk<sub>3</sub>, respectively, at 6'-O position.

Ginsenoside Rg<sub>6</sub>, Rk<sub>1</sub>, Rk<sub>2</sub>, Rk<sub>3</sub>, Rs<sub>5</sub>, and Rs<sub>7</sub> are the  $\Delta^{20(21),24}$ -diene -dammarane compounds,

whose C-20, 21 and 22 carbons were appeared at *ca.* 155.5, 108.2 and 33.9 ppm, respectively, in the  $^{13}\text{C}$  NMR. In their  $\Delta^{20(22),24}$ -diene isomers, namely  $\text{F}_4$ ,  $\text{Rg}_5$ ,  $\text{Rh}_3$ ,  $\text{Rh}_4$ ,  $\text{Rs}_4$  and  $\text{Rs}_6$ , C-20, 21 and 22 carbons were appeared at *ca.* 140.1, 13.1 and 123.2 ppm, respectively.

These sets of isomers were not separated in silica column.  $\text{AgNO}_3$ -impregnated silica was used to separate the isomers. Figure 1 and 2 show the structure of isolated ginsenosides.  $^{13}\text{C}$ -NMR spectral data of isolated ginsenosides are summarized in Table I and III.  $^1\text{H}$ -NMR spectral data of isolated ginsenosides are summarized in Table II and IV. All peaks were identified by the reference of reported data and DEPT, C-H COSY, C-H long range COSY, and decoupling experiments. Note that the reported  $^{13}\text{C}$ -NMR data for the ginsenoside  $\text{Rh}_4$  are modified by extensive reexamination.

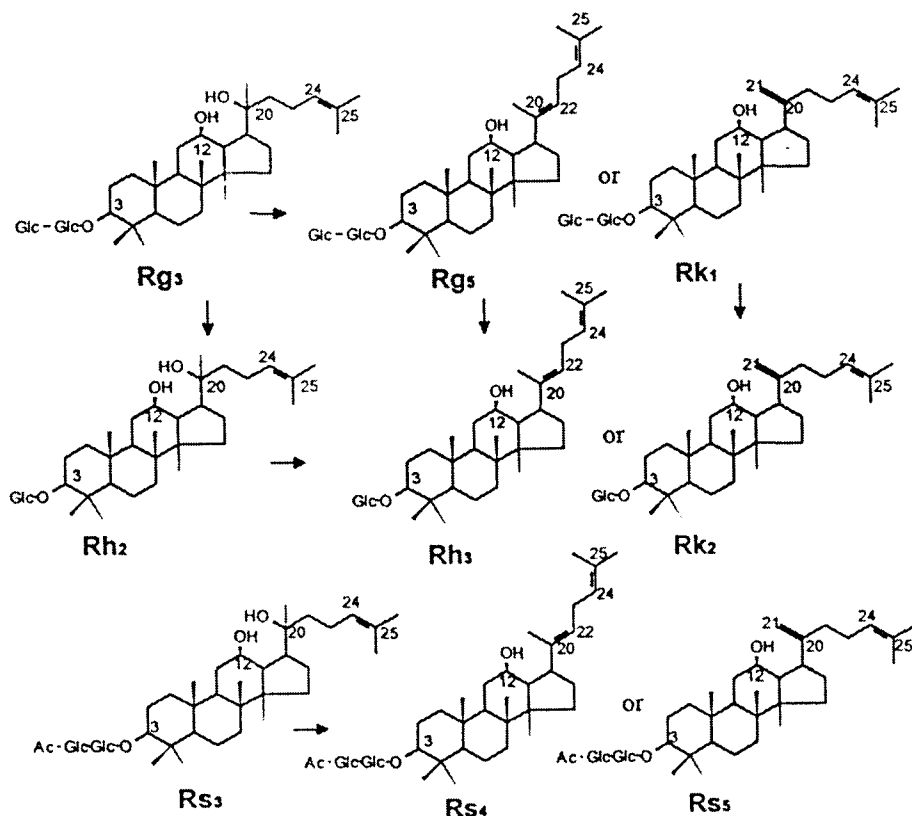


Fig.1. Conversion of protopanaxadiol saponins by heat treatment

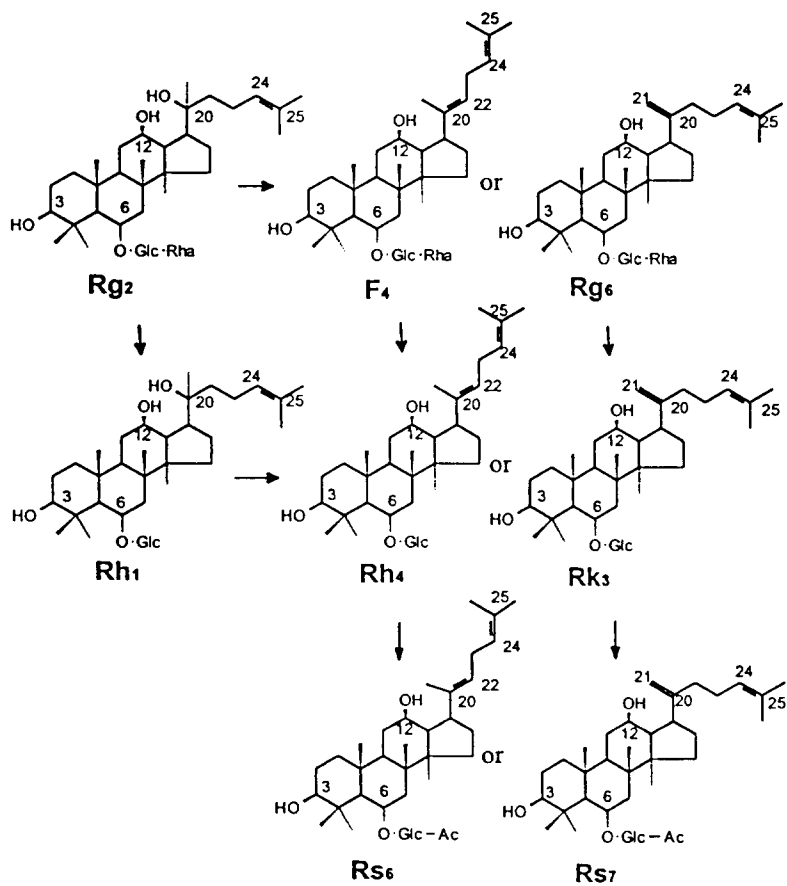


Fig.2. Conversion of protopanaxatriol saponins by heat treatment

Table I.  $^{13}\text{C}$ -NMR data of protoanaxadiol saponins

C No.	<sup>a</sup> Rh <sub>2</sub>	<sup>a</sup> 20(S) -Rg <sub>3</sub>	<sup>a</sup> 20(R) -Rg <sub>3</sub>	<sup>a</sup> Rg <sub>5</sub>	<sup>a</sup> Rk <sub>1</sub>	<sup>a</sup> Rh <sub>3</sub>	<sup>a</sup> Rk <sub>2</sub>	<sup>a</sup> Rs <sub>3</sub>	<sup>a</sup> Rs <sub>4</sub>	<sup>a</sup> Rs <sub>6</sub>
1	39.12	39.07	39.13	39.17	39.30	39.22	39.44	38.99	39.29	39.29
2	27.05	26.66	26.72	28.00	26.75	28.10	26.92	26.78	26.79	26.79
3	88.78	88.89	88.91	88.82	88.95	88.72	88.95	89.14	89.20	89.21
4	39.66	39.64	39.70	40.14	39.72	40.22	39.86	39.94	39.75	39.74
5	56.35	56.30	56.36	56.29	56.43	56.35	56.57	56.34	56.46	56.47
6	18.43	18.37	18.44	18.33	18.45	18.41	18.63	18.40	18.48	18.48
7	35.13	35.10	35.17	35.24	35.36	35.29	35.50	35.81	35.37	35.37
8	40.00	39.93	40.00	39.60	40.21	39.65	40.38	36.87	40.29	40.22
9	50.38	50.31	50.37	50.66	48.23	50.72	51.02	50.33	50.79	50.88
10	36.94	36.85	36.91	36.91	37.03	37.02	37.23	39.67	37.07	37.06
11	<sup>a</sup> 32.02	<sup>a</sup> 31.95	<sup>a</sup> 32.15	32.10	32.60	32.19	32.88	31.97	32.20	32.60
12	70.96	70.95	70.88	72.49	72.47	72.51	72.59	70.94	72.61	72.48
13	49.54	48.50	49.21	50.33	52.49	50.41	52.63	48.50	51.04	52.48
14	51.69	51.64	51.76	50.91	51.21	50.98	51.38	51.64	50.90	51.21
15	<sup>a</sup> 31.32	<sup>a</sup> 31.28	<sup>a</sup> 31.41	32.54	32.67	32.59	32.78	31.27	32.63	32.66
16	26.70	26.78	26.63	26.64	30.77	26.70	30.95	26.71	28.83	30.76
17	54.77	54.71	50.62	50.80	50.86	50.86	48.44	54.75	50.44	48.24
18	<sup>b</sup> 16.77	<sup>b</sup> 16.54	<sup>b</sup> 16.59	16.35	16.45	16.42	15.97	16.94	15.84	15.82
19	<sup>b</sup> 15.61	<sup>b</sup> 15.76	<sup>b</sup> 15.82	16.49	15.80	16.75	16.64	16.30	16.45	16.43
20	72.94	72.93	72.97	140.06	155.55	140.12	155.71	72.89	140.19	155.55
21	26.83	27.00	22.60	13.07	108.15	13.13	108.28	27.01	13.16	108.15
22	35.88	35.81	43.25	123.21	33.89	123.78	34.01	35.10	123.51	33.87
23	22.97	22.93	22.76	27.35	27.08	27.41	27.22	22.93	27.45	27.08
24	126.30	126.24	126.05	123.54	125.33	124.53	125.52	126.25	123.8	125.33
25	130.73	130.71	130.77	131.16	131.21	131.22	131.38	130.69	131.26	131.20
26	<sup>b</sup> 25.78	<sup>b</sup> 25.77	<sup>b</sup> 25.62	25.60	25.74	25.66	25.92	25.75	25.67	25.74
27	17.66	17.63	17.70	17.66	17.74	17.68	17.91	17.62	17.71	17.74
28	28.14	28.05	28.11	28.73	28.11	28.80	28.32	27.95	28.01	28.01
29	<sup>b</sup> 16.34	<sup>b</sup> 16.29	<sup>b</sup> 16.37	15.72	16.58	15.78	16.95	15.77	16.45	16.43
30	<sup>b</sup> 17.65	<sup>b</sup> 16.95	<sup>b</sup> 17.30	16.92	16.98	17.00	17.16	16.39	17.03	16.98
1'	106.92	105.03	105.12	105.00	105.09	106.93	107.16	104.84	104.9	104.89
2'	75.76	83.27	83.45	83.31	83.45	75.75	75.98	84.24	84.29	84.26
3'	<sup>c</sup> 78.72	<sup>c</sup> 78.27	<sup>c</sup> 78.25	78.13	78.19	78.72	78.95	78.07	78.07	78.06
4'	71.65	71.54	71.62	71.50	71.65	71.83	72.07	71.30	71.02	71.02
5'	<sup>c</sup> 78.34	<sup>c</sup> 77.03	<sup>c</sup> 77.14	77.82	77.96	78.34	78.57	77.87	77.92	77.92
6'	63.05	62.76	62.64	62.58	62.76	63.04	63.27	62.75	62.84	62.84
1''		105.92	106.05	105.91	106.01			106.13	106.17	106.14
2''		77.89	77.95	77.00	77.08			76.68	76.73	76.71
3''		78.27	<sup>c</sup> 78.33	78.21	78.34			78.49	78.52	78.52
4''		71.59	71.64	71.53	71.72			70.94	71.42	71.42
5''		78.03	<sup>c</sup> 78.11	77.98	78.06			70.29	75.36	75.35
6''		62.65	62.69	62.73	62.87			64.70	64.74	64.74
CO								170.96	170.97	170.96
CH <sub>3</sub> CO								20.85	20.9	20.88

a), b) and c) assignments may be interchangeable within the same vertical column.

<sup>a</sup> Data of Rh<sub>2</sub>, Rg<sub>3</sub>, Rg<sub>5</sub>, Rh<sub>3</sub>, and Rs<sub>3</sub> are from ref. [5], [6], [7], [4], and [8], respectively.

<sup>§</sup> Spectra were recorded in pyridine-d<sub>5</sub> at 125 MHz.

**Table II. <sup>1</sup>H-NMR data of protopanaxadiol saponins**

H No.	<sup>a</sup> Rh <sub>2</sub>	<sup>b</sup> Rg <sub>3</sub>	<sup>a</sup> Rg <sub>5</sub>	<sup>a</sup> Rh <sub>3</sub>	<sup>b</sup> Rs <sub>4</sub>
3	3.92 dd-like	3.26 m	3.26 dd(4.0, 11.6)	3.27 dd(11.7,4.3)	3.28 dd(11.6,4.3)
5				0.64 dd(11.0,2.0)	0.73 d(12.0)
12	3.35 ddd-like	3.80 m	3.90 br.s	3.81 ddd-like	3.92 m
17		2.80 m		2.67 ddd-like	2.80 m
18	0.976 <sup>a</sup> s	1.00 s	1.09 s	0.91 s	1.18 s
19	1.005 <sup>a</sup> s	0.95 s	1.01 s	0.90 s	1.05 s
21	1.384 s	1.63 s	1.81 s	1.72 s	1.82 s
24	5.31 t(6.8)	5.49 br.t(6.0)	5.21 t(6.9)	5.12 tq(7.1,1.2)	5.23 t(6.9)
26	1.034 <sup>a</sup> s	1.58 s	1.62 s	1.54	1.63 s
27	1.289 <sup>a</sup> s	1.51 s	1.58 s	1.50 s	1.59 s
28	1.646 <sup>a</sup> s	2.12 s	1.27 s	1.21 s	1.34 s
29	1.689 <sup>a</sup> s	1.80 s	0.81 s	0.71 s	0.85 s
30	0.816 <sup>a</sup> s	0.99 s	0.95 s	0.85 s	0.97 s
1'	4.93 d(7.6)	4.89 d(7.4)	4.90 d(7.4)	4.82 d (11.9)	4.89 d(7.3)
1''		5.32 d(6.8)	5.33 d(7.6)		5.31 d(7.7)
CH <sub>3</sub> CO					2.05

H No.	<sup>a</sup> Rs <sub>3</sub>	<sup>b</sup> Rk <sub>1</sub>	<sup>b</sup> Rk <sub>2</sub>	<sup>b</sup> Rs <sub>5</sub>
3	3.27 dd(4.5,11.6)	3.23 dd(11.76,4.37)	3.40 dd(11.5,4.5)	3.24 dd(11.58,4.43)
5		0.62 d(11.86)	0.77 d(10.5)	0.67 d(11.86)
12	3.94 m	3.89 m	3.93 m	3.90 m
17		2.77 m	2.85 m	2.79 m
18	0.95 s	0.95 s	1.04 s	0.99 s
19	1.12 s	0.72 s	0.82 s	0.77 s
21	1.42 s	4.86, 5.13	4.94, 5.19	4.88, 5.14
24		5.23 d(6.68)	5.31 t(6.9)	5.24 d(7.75)
26	1.64 s	1.61 s	1.68 s	1.62 s
27	1.61 s	1.49 s	1.62 s	1.57 s
28	1.34 s	1.31 s	1.33 s	1.26 s
29	0.81 s	1.02 s	1.02 s	1.05 s
30	0.96 s	0.91 s	0.99 s	0.93 s
1'	4.92 d(7.6)	4.83 d(7.53)	4.95 d(7.82)	4.84 d(7.36)
1''	5.33 d(7.7)	5.33 d(7.71)		5.24 d(7.75)
CH <sub>3</sub> CO				2.02

a) assignments may be interchangeable within the same vertical column.

The values in parenthesis are coupling constants in Hz.

\* Data of Rh<sub>2</sub>, Rg<sub>5</sub>, Rh<sub>3</sub>, and Rs<sub>3</sub> are from ref. [5], [7], [4], and [8], respectively.

<sup>b</sup> Spectra were recorded in pyridine-d<sub>5</sub> at 500 MHz.

**Table III.**  $^{13}\text{C}$ -NMR data of protopanaxatriol saponins

C No.	$^{20}(\text{R})$ -Rh <sub>1</sub>	$^{20}(\text{S})$ -Rh <sub>1</sub>	$^{\text{a}}\text{Rg}_2$	$^{\text{a}}\text{F}_4$	$^{\text{a}}\text{Rg}_6$	$^{\text{a}}\text{Rh}_4$	$^{\text{a}}\text{Rk}_3$	$^{\text{a}}\text{Rs}_6$	$^{\text{a}}\text{Rs}_7$
1	39.6	39.4	39.4	39.5	39.6	39.44	39.50	39.48	39.58
2	27.8	27.9	28.0	27.8	27.8	27.80	27.92	27.89	27.94
3	78.5	78.6	78.3	78.4	78.3	78.52	78.56	78.51	78.61
4	40.2	40.3	40.2	40.1	40.0	40.27	40.37	40.29	40.32
5	61.3	61.4	60.7	60.9	60.9	61.36	61.44	61.40	61.49
6	77.9	78.0	74.6	74.4	74.5	79.97	80.05	79.69	79.72
7	45.0	45.2	45.7	46.2	46.2	45.22	45.31	45.60	45.66
8	41.0	41.1	41.0	41.4	41.4	41.25	41.26	41.42	41.46
9	50.1	50.2	50.1	50.1	48.2	50.50	50.64	50.55	50.70
10	39.6	39.6	39.4	40.0	39.7	39.66	39.71	39.75	39.82
11	<sup>a</sup> 32.0	<sup>a</sup> 32.0	30.6	32.2	32.7	32.18	32.73	32.31	32.80
12	70.8	71.0	70.3	70.3	72.3	72.51	72.42	72.49	72.49
13	48.7	48.2	48.5	50.7	52.2	50.59	52.07	50.69	52.21
14	51.6	51.6	51.3	50.9	51.2	50.77	51.13	50.89	51.27
15	<sup>a</sup> 31.6	<sup>a</sup> 31.1	30.6	32.6	32.6	32.47	32.50	32.69	32.71
16	26.6	27.2	26.5	27.1	27.1	28.74	30.71	28.77	30.76
17	50.4	54.7	51.8	52.0	50.3	50.32	48.27	50.39	48.22
18	<sup>b</sup> 17.3	<sup>b</sup> 17.4	17.4	17.7	17.8	17.31	17.33	17.36	17.41
19	<sup>b</sup> 17.6	<sup>b</sup> 17.6	17.4	17.8	17.7	17.67	17.73	17.72	17.78
20	73.0	73.0	73.2	140.1	155.5	140.01	155.42	140.02	155.47
21	22.6	26.8	22.5	27.5	108.1	13.07	108.11	13.16	108.24
22	43.1	35.8	35.8	123.5	33.8	123.42	33.70	123.17	33.99
23	22.6	23.0	23.3	30.0	30.7	27.38	27.02	27.43	27.12
24	125.9	126.3	125.7	125.4	125.4	123.78	125.33	123.83	125.37
25	130.7	130.6	130.9	131.3	131.3	131.18	131.18	131.22	131.25
26	25.8	25.8	25.7	25.8	25.8	25.64	25.71	25.68	25.77
27	17.6	17.6	17.6	17.6	17.6	17.67	17.33	17.70	17.78
28	31.6	31.7	32.0	32.6	32.2	31.63	31.70	31.54	31.59
29	<sup>b</sup> 16.3	<sup>b</sup> 16.4	17.1	17.0	16.9	16.27	16.34	16.50	16.51
30	<sup>b</sup> 17.0	<sup>b</sup> 16.8	17.1	17.2	17.2	16.73	16.73	16.96	17.00
1'	105.7	105.9	101.6	101.8	101.9	105.87	106.00	105.90	105.92
2'	75.3	75.4	79.1	79.5	79.4	75.34	75.45	75.34	75.41
3'	<sup>c</sup> 80.0	<sup>c</sup> 80.0	78.0	78.4	78.4	79.50	79.65	79.20	79.22
4'	71.7	71.8	72.1	72.6	72.7	71.71	71.82	71.37	71.47
5'	<sup>c</sup> 79.5	<sup>c</sup> 79.5	78.0	78.4	78.7	77.98	78.12	75.08	75.17
6'	62.9	63.1	62.9	63.2	63.2	62.96	63.06	65.12	65.17
1''			102.0	102.0	101.9				
2''			72.1	72.3	72.4				
3''			72.1	72.4	72.4				
4''			73.8	74.2	74.2				
5''			69.3	69.5	69.5				
6''			18.6	18.8	18.7				
CO							170.88	170.86	
CH <sub>3</sub> CO							20.93	20.93	

a), b) and c) assignments may be interchangeable within the same vertical column.

<sup>a</sup> Data of Rh<sub>1</sub>, Rg<sub>2</sub>, F<sub>4</sub>, and Rg<sub>6</sub> are from ref. [9], [10], [10], and [11], respectively.

<sup>§</sup> Spectra were recorded in pyridine-d<sub>5</sub> at 125 MHz.



**Table IV. <sup>1</sup>H-NMR data of protopanaxatriol saponins**

H No.	<sup>§</sup> Rh <sub>4</sub>	<sup>*</sup> F <sub>4</sub>	<sup>§</sup> Rs <sub>6</sub>	<sup>*</sup> Rg <sub>6</sub>	<sup>§</sup> Rk <sub>3</sub>	<sup>§</sup> Rs <sub>7</sub>
3	3.51 dd(11.5,4.6)		3.50 dd(11.5,4.5)	3.55 m	3.56 dd(11.5,4.5)	3.51 d(11.4)
6	4.41 m		4.41 m	4.76 m	4.45 m	4.43 m
7	2.50 dd(12.6,2.8)		2.49 d(10.0)		2.53 dd(12.8,3.3)	2.51 dd(12.5,3.0)
12	3.91 m		4.01 m	3.97 m	3.98 m	3.93 m
17	2.70 m		2.77 m		2.77 m	2.82 m
18	1.21 s		1.29 s		1.30 s	1.31 s
19	1.02 s	0.98 s	1.05 s		1.05 s	1.08 s
21	1.08 s	1.81 s	1.83 s	4.95, 5.17	4.97, 5.23	4.92, 5.16
24	5.20 t(7.1)	5.25 br.t(6.0)	5.21 t(6.8)	5.35 t(6.5)	5.34 t(6.8)	5.29 m
26	1.61 s	1.64 <sup>a)</sup> s	1.61 s		1.74 s	1.67 s
27	1.60 s	1.59 <sup>a)</sup> s	1.56 s		1.67 s	1.61 s
28	2.05 s	1.27 s	2.05 s		2.05 s	2.02 s
29	1.56 s	2.14 s	1.55 s		1.60 s	1.55 s
30	0.80 s	1.31 s	0.95 s		0.93 s	0.98 s
1'	5.00 d(7.8)	5.28 d(7.5)	5.05 d(7.7)	5.33 d(6.7)	5.01 d(7.8)	5.04 d(7.7)
1''		4.81 d(2.4)		6.56 s		
CH <sub>3</sub> CO			2.06 s			2.06 s

a) assignments may be interchangeable within the same vertical column.

The values in parenthesis are coupling constants in Hz.

\* Data of F<sub>4</sub> and Rg<sub>6</sub> are from ref. [10] and [11], respectively.

§ Spectra were recorded in pyridine-d<sub>5</sub> at 500 MHz.

#### *Analysis of ginsenosides by HPLC/ELSD*

HPLC methods reported so far for the analysis of ginsenosides were not sufficient to separate the saponins in a new processed ginseng since it contains less polar saponins as a major constituents. Amino column with evaporative light scattering detection (ELSD) method was very successful to separate relatively polar ginsenosides, however the method was not adequate to separate less polar saponins. Furthermore amino column cannot separate 20(S)-ginsenosides from corresponding 20(R)-isomers, and  $\Delta^{20(21),24}$  ginsenosides from corresponding  $\Delta^{20(22),24}$  isomers. Reverse phase HPLC using octadecylsilyl (ODS) column with gradient elution was successful to separate major saponins in processed ginseng. Fig. 3 is a chromatogram of standard mixture of ginsenosides. 20(S)-ginsenosides and  $\Delta^{20(21),24}$ -ginsenosides are eluted earlier than corresponding 20(R)- and  $\Delta^{20(22),24}$ -isomers, respectively.

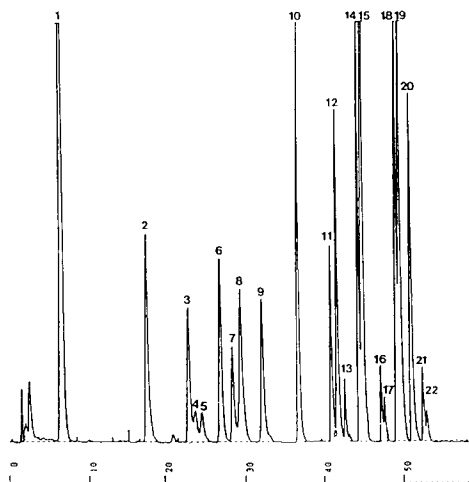


Fig. 3. Chromatogram of standard mixture of ginsenosides

Column:  $\mu$ -Bondapak C<sub>18</sub>(10 $\mu$ m, 3.9mm  $\phi$  x 300mm), Detection: evaporative light scattering detector  
 Mobile phase: A: CH<sub>3</sub>CN/0.6% HAc = 25/75, B: CH<sub>3</sub>CN

100% A  $\rightarrow$  90% A (100%B) linear gradient for 30 min

90% A  $\rightarrow$  0% A (100%B) linear gradient for 40 min

1: Rg<sub>1</sub>+Re, 2: Rf, 3: 20(S)-Rh<sub>1</sub>+Rg<sub>2</sub>, 4: 20(R)-Rg<sub>2</sub>, 5: 20(R) Rh<sub>1</sub>, 6: Rh<sub>2</sub>, 7: Rb<sub>1</sub>, 8: Rg<sub>3</sub>, 9: Rb<sub>2</sub>, 10: Rd, 11: Rg<sub>4</sub>, 12: F<sub>4</sub>,  
 13: Rh<sub>3</sub>, 14: 20(S)-Rg<sub>3</sub>, 15: 20(R)-Rg<sub>3</sub>, 16: 20(S)-Rg<sub>5</sub>, 17: 20(R) Rg<sub>5</sub>, 18: Rk<sub>1</sub>, 19: Rg<sub>6</sub>, 20: 20(S)-Rb<sub>2</sub>, 21: Rf<sub>2</sub>, 22: Rg<sub>7</sub>

### Change of ginsenosides by heat treatment

Fig. 4 shows the change of ginsenosides on heat processing. Chromatogram (a) is from raw ginseng (fine root ginseng) without processing. Ginsenoside Rg<sub>1</sub>, Re, Rb<sub>1</sub>, Rc, Rb<sub>2</sub>, and Rd were the predominant saponins. Steamed ginseng at 100 °C for 3 hours, which is similar to red ginseng preparation condition, showed less polar saponins of Rg<sub>6</sub>, F<sub>4</sub>, Rh<sub>4</sub>, 20(S)-Rg<sub>3</sub>, 20(R)-Rg<sub>3</sub>, Rk<sub>1</sub> and Rg<sub>5</sub> as minor constituents as in chromatogram (b) of Fig. 4. The contents of these less polar saponins were increased in steamed ginseng at 110 °C for 3 hrs, and eventually they became major constituents in processed ginseng at 120 °C.

What happens to the polar ginsenosides on heat treatment are 1) loss of glycosyl moiety at C-20 position (formation of Rg<sub>3</sub>, Rg<sub>2</sub>), 2) loss of H<sub>2</sub>O at C-20 position, which results in two isomers of  $\Delta^{20(21),24}$  and  $\Delta^{20(22),24}$ -diene-dammaranes (formation of Rg<sub>5</sub>, Rk<sub>1</sub>, F<sub>4</sub>, Rg<sub>6</sub>), 3) loss of one sugar moiety at C-3 or C-6 position. (formation of Rh<sub>2</sub>, Rh<sub>3</sub>, Rk<sub>2</sub>, Rh<sub>1</sub>, Rh<sub>4</sub>, Rk<sub>3</sub>), 4) 6'-O-acetylation of sugar (formation of Rs<sub>3</sub>, Rs<sub>4</sub>, Rs<sub>5</sub>, Rs<sub>6</sub>, Rs<sub>7</sub>). Consequently, the content of polar ginsenosides are decreased while that of less polar ginsenosides are increased.

Fig. 5 summarizes the effect of processing temperature on the content of ginsenosides. Ginsenosides can be classified as three groups. First, polar ginsenosides whose contents were increased until 105 °C and decreased thereafter, eventually almost disappeared at 130 °C. Ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, and Rg<sub>1</sub> belong to first group. Second, medium polar ginseno-

sides whose contents were increased until 125 °C and decreased thereafter. Ginsenoside Rg<sub>2</sub>, Rh<sub>1</sub>, and Rf are belong to second group. Third, less polar ginsenosides whose contents were gradually increased until 130 °C. Ginsenoside Rg<sub>3</sub>, Rg<sub>5</sub>, Rg<sub>6</sub>, Rk<sub>1</sub>, Rk<sub>2</sub>, Rk<sub>3</sub>, Rh<sub>3</sub>, Rh<sub>4</sub>, F<sub>4</sub>, Rs<sub>3</sub>, and Rs<sub>4</sub> belong to third group. The content of ginsenosides in the third group was decreased over 140 °C (data not shown).

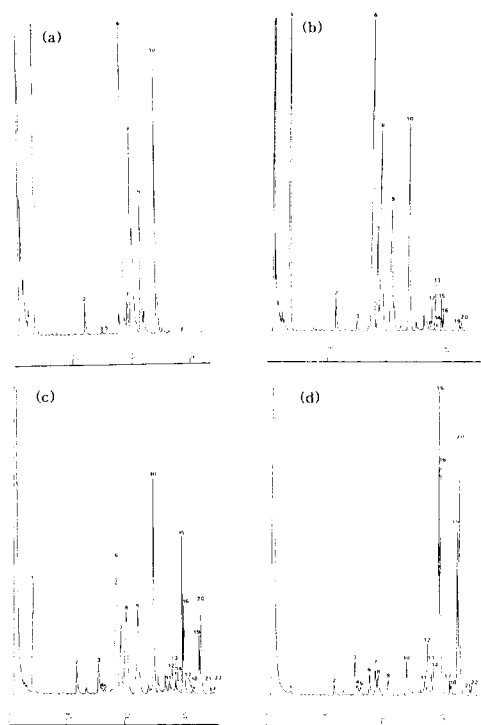


Fig. 4 Chromatograms of (a) non processed fine root ginseng, (b) heat processed fine root ginseng at 100°C, (c)110°C, (d)120°C by ODS-HPLC/ELSD

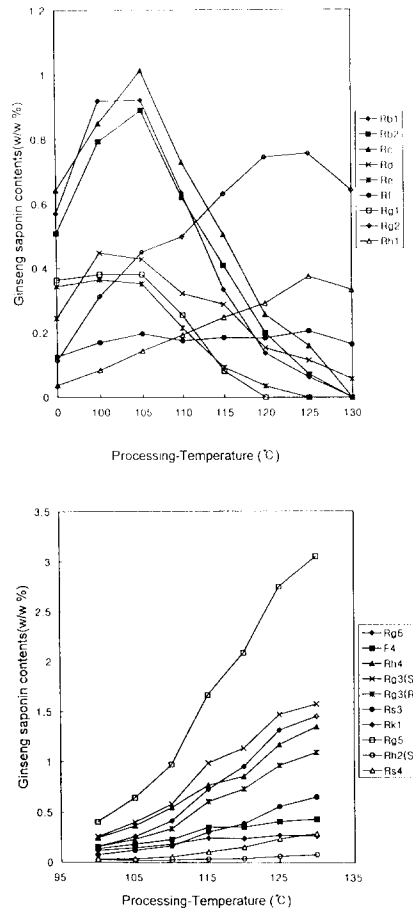
Analytical condition and peak identify are same as in Fig.3

### *Biological activity of processed ginseng*

Fig. 6 shows the antioxidant activity (radical scavenging activity) of processed ginseng analyzed by DPPH (diphenylpicrylhydrazyl) method[15]. Processed ginseng at 120 °C showed decidedly higher activity than that shown in fresh ginseng or in processed ginseng at 100 °C.

Fig. 7 shows the vasodilation effect of the processed ginseng. As it can be seen from the results, although all ginseng extract exhibited dose-dependent relaxation in aortae, processed ginseng at 120 °C definitely showed stronger activity even at a lower dose than raw ginseng or processed ginseng at

Fig. 5 Effect of processing temperature on the contents of ginseng saponins.



lower temperature. The vasodilation effect of the processed ginseng was not observed in blood vessel from which endothelial cell is removed. Accordingly, it shows that the vasodilation activity of the processed ginseng in aortae is endothelium-dependent. Further study revealed that ginsenoside Rg<sub>3</sub>, Rg<sub>5</sub> and Rk<sub>1</sub> are the active principles. These ginsenosides released NO from endothelium cell.

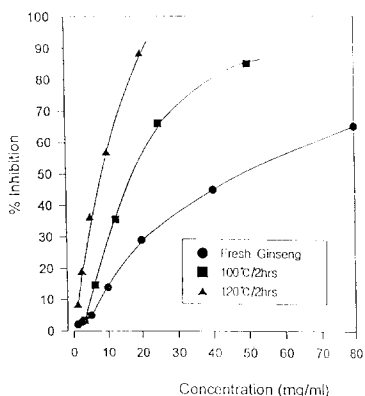


Fig. 6 Antioxidant activity of processed ginseng

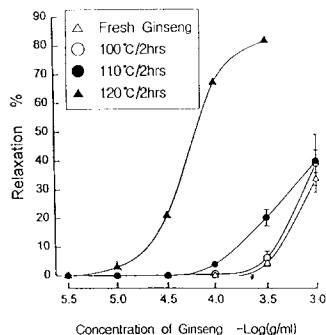


Fig. 7 Concentration-response curve of various ginseng extracts in rat aortic rings with endothelium. Relaxations were expressed as a percentage of relaxation to maximum contraction of phenylephrine( $10^{-6}$  M).

In addition, new processed ginseng exhibited cancer chemoprevention activity *in vitro* and *in vivo* model studies. The new processed ginseng greatly reduced nephrotoxicity induced by an anticancer agent, cisplatin. The processed ginseng reduced neurotoxicity induced by glutamate in primary cultures of rat cortical cells. These activities are more potent in processed ginseng than in raw ginseng. (data not shown)

Less polar ginsenosides isolated from processed ginseng reduced platelet aggregation induced by collagen, and inhibited tumor cell growth analyzed by radioactive thymidine incorporation study and MTT assay. Less polar ginsenosides induced apoptosis in tumor cell. (data not shown)

## Conclusion

Steaming of ginseng at 120~130 °C for 2~3 hrs yielded a new processed ginseng having increased biological activities. The trace components of a red ginseng are significantly increased as well as novel components are produced. Biological activities tested so far are greatly increased compared to raw ginseng which is not processed.

The results demonstrate the applicability of the processed ginseng as preventing or treating agent in diseases such as cancer, hypertension, sexual dysfunction due to circulatory disorder, etc.

Since the chemical constituents and biological activities of the new processed ginseng are quite different from those of white ginseng or red ginseng, we designated it as “*sun ginseng* (仙蔘)”. Further studies on the chemical constituents and biological activities of “*sun ginseng*” are under progress.

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