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Chemical Composition of *Panax Ginseng*-Leaf Tea

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Abstract□Chemical composition was determined to evaluate the quality of *Panax ginseng*-leaf tea over green teas. Ginseng-leaf tea was shown to contain higher contents of soluble matter, ascorbic acid and lower contents of tannins, as compared to tea leaves. The profiles of ginsenoside and sugar of ginseng-leaf tea were noticeably different from those of ginseng roots and the sample maintained high levels of these components under the manufacturing process. Total unsaturated fatty acids and free amino acids were estimated to be decreased in ginseng-leaf tea as compared to those of ginseng leaves. The compositions of amino acids and minerals in ginseng-leaf tea were similar to those of tea leaves and glutamic acid, aspartic acid, leucine, calcium, potassium, sodium, and copper were found to be major components.

Key words□*Panax ginseng*-leaf tea, chemical composition, quality evaluation

Introduction

Ginseng is one of the most famous plant drugs and health foods in oriental countries. Since the first finding of the presence of saponin in *Panax* spp. by Garriques,[1] a number of studies have determined the chemical characteristics of dammarane-type saponins(more than 20 ginsenosides) of ginseng roots and their individual activities from the parmacological and biological points of view.[2-10] These scientific demonstrations of the values of ginseng have led to a significant increase in its consumption in recent years.[11,12]

In ginseng products it is generally recognized that the higher the saponin content, the better the quality. Despite the presence of higher amounts of dammarane-type saponins in ginseng leaves than its roots, practical utilization of the leaves has been restricted.[13,14]

To meet consumer demand and preference, various ginseng products have been developed, such as extracts, drinks, tea, powder, capsule and tablet, which are prepared by using ginseng materials or along with flavored additives. Presently, more than 200 items of 40 types of ginseng products are commercially available in over 70 countries around the world, but further efforts are required for the development of new products and improvement of their qualities.

Since chemical research on ginseng leaves was initiated by Shibata *et at.*,[15] the main components of the leaves have been investigated in view of pharmacological and compositional properties and compared with those of ginseng roots.[14-16] However, in connection with the possible utilization of by product of the ginseng plant, the leaves were only subjected to several attempts in the preparation of leaf tea and leaf protein concentrate.[17-19]

The present study intends to determine the chemical constituents of ginseng-leaf tea for the purpose of evaluating its quality over green teas.

Materials and Methods

Materials: Ginseng-leaf tea (*Panax ginseng* C.A. Meyer) used in this study was prepared according

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to the commercial procedure by which most exportable products are being manufactured in the ginseng products industry in Korea. Fresh ginseng leaves (4 year old plant) harvested in September 1990 from the field at a local producing district, Kanghwa, were first washed with water, selected and then dried in the shade before steaming for 15 min. The steamed leaves were air-dried at around 50℃ to contain below 10% of moisture content. The dried samples, which are normally milled for a commercial packaging, were reduced to powder (60 mesh) by a Waring blendor (Waring products Co.) for chemical analyses.

Prosimate analyses: Proximate compositions of ginseng-leaf tea were determined according to standard AOAC methods.[20] Measurements for reducing sugar and starch were based upon the modified Somogyi method.[21] Water-soluble matter and butanol-soluble extract of the sample were gravimetrically determined.[22,23] The spectrophotometric methods (Baush & Lomb, spectronic 710) were applied for determining tannic acid,[24] ascorbic acid,[25] chlorophyll and pheophytin.[26] Samples were shaken for 20 min with 5 times their volume of deionized water and the pH of the supernatant was determined using a Corning Model 120 pH meter. The supernatant was neutralized with 0.05 N NaOH for the determination of titratable acidity.

Determination of saponins and free sugars: Ginsenosides and free sugars were determined in ginseng-leaf tea with some modifications of an earlier method.[22] Ginseng-leaf tea was extracted for 4 hrs with 80% ethanol at 80℃ and the same procedure was repeated 4 times for the sample. The combined solvent was removed in a rotary evaporator, and the residue was dissolved in water. The aqueous solution was extracted with diethyl ether to remove the residual lipid components and pigments and then with a saturated aqueous n-butanol solution. The butanol layer was concentrated *in vacuo* and used for ginsenoside analysis using a high-performance liquid chromatograph (HPLC). On the other hand, the aqueous solution was set aside for sugar analysis after concentration. HPLC was performed on a BioRad 402 system equipped with a refractive index detector and a Lichrosorb-NH_2 column (5 μm stainless steel, 20 cm); a mobile phase made up of acetonitrile-water-1-butanol (43 : 7 : 5, v/v/v) was used as eluant. Reference ginsenosides were used to prepare a standard curve of the major saponin components, i.e., ginsenosides $-Rb_1$, $-Rb_2$, $-Rc$, $-Rd$, $-Re$, $-Rf$, $-Rg_1$ and $-Rg_2$.

HPLC was also used for sugar analysis with the aqueous solution from above. The chromatograph was operated under the same conditions as for the saponin alalysis except that the mobile phase consisted of acetonitrile-water (43 : 8, v/v).

Determination of fatty acids: Grude lipids were extracted from the sample with a solvent system of chloroform-methanol (2 : 1, v/v) and purified as described by Folch *et al.*[27] Fatty acid esters were obtained by methylesterification of the purified lipids using BF_3-methanol following hydrolysis by 0.5 N sodium hydroxide[28] and analyzed with a gas chromatograph (Varian Aerograph Model 3700) equipped with a flame ionization detector. A fused silicagel capillary column (30 m×0.32 mm i.d., 0.2 μm film thickness) coated with SP-2340 was used for methyl ester separation. The column oven temperature was programmed linearly from 150℃ (5 min) to 200℃ (20 min) at an increasing rate of 4℃ per min. The temperature of injector and detector was maintained at 240℃. Flow rate of the nitrogen carrier gas was 1.0 m*l* per min and split ratio was 1 : 30. The peaks were identified by comparing retention time to that of a standard mixture of known fatty acid methyl esters. Peak areas were integrated using Shimadzu C-RIA chromatopac and the percentage of total fatty acids was determined.

Determination of amino acids: Analyses of total amino acids were caried out after HCl hydrolysis. Hydrochloric acid (6 N) was added to the sample in a pyrex tuve that was then sealed *in vacuo*. It was heated at 100℃ for 24 hrs to allow for a complete hydrolysis. After cooling, the solution was filtered and evaporated to dryness under reduced pressure. Subsequently, 0.5 m*l* of 0.01 N NaOH solution was added to the sample and allowed to stand at room temperature for 4 hours. Oxidation of cysteine into cystine took place during that period. The volume was adjusted to 2 m*l* by the addition of 0.02 N HCl. The final solution was then injected into an

amono acid analyzer (Hitachi Model 835-50).

Free amino acids were extracted three times with 75% ethanol for 20 minutes. The combined extracts were evaporated by heat to obtain an aqueous solution to which an equal volume of diethyl ether was added and shaken to remove the residual lipids from the sample. After evaporating the aqueous layer, a 0.01 N NaOH solution was added to allow for the oxidation of cysteine into cystine. A solution of 0.02 N HCl was added to obtain an appropriate volume for injection. Tryptophan was not measured in this study.

Determination of Minerals: Nine different elements were analyzed using inductively coupled plasma spectrometer (ICP, ARL, Model 3510) following established wet-digestion procedures.[29]

Results and Discussion

Table 1. Chemical constituents of ginseng-leaf tea[a]

Constituents	Content
Moisture (%)	9.92± 0.24
Protein (%)	14.96± 1.45
Fat (%)	3.76± 0.40
Ash (%)	7.42± 0.82
Fiber (%)	9.80± 1.40
Reducing sugar (%)	2.10± 0.36
Starch (%)	34.76± 1.20
Soluble matter (%)	32.16± 2.46
Crude saponin (%)	14.56± 1.96
Tannic acid (%)	3.00± 1.84
Ascorbic acid (mg%)	38.20± 2.57
Chlorophyll (mg%)	314.00± 9.40
Pheophytin (mg%)	338.70± 7.26
pH	6.10± 0.12
Acidity	27.48± 1.46

[a] Mean± standard deviation of triplicate determinations.

Proximate constituents: Tea qualities are largely determined by several characteristics of the products, such as appearance, color, extract color, smell and taste. More than 500 chemical constituents are known to be included in tea, however those compounds which are related to the pharmacological actions and unique taste of the tea are polyphenols, amino acids, flavonoids, vitamins, caffeine, polysaccharides, etc.[30] Chemical constituents of ginseng-leaf tea are shown in Table 1. Proximate compositions of the sample were similar to those of raw ginseng leaves and crude saponin (14.56%) showed a higher percentage than other parts of ginseng (1.07~6.75%).[18,31] Ginseng-leaf tea contained more soluble matter (32.16%) and ascorbic acid (38.20 mg%) than tea leaves, 23~26% and 5~8 mg% respectively.[32] Furthermore, a lower content of tannin was observed in the sample than tea leaves,[33,34] suggesting that it will contribute to a less-astringent and attractive taste of ginseng-leaf tea, especially when it combines with the characteristic taste of saponins.

Saponins and sugars: Ginseng is easily distinguished from other similar herbs by means of compositional difference of ginsenosides, which is a primary reason why the saponin has been a mysterious subject for the intensive scientific research over centuries.[35-37] Major ginsenosdies detected in ginseng-leaf tea are presented in Table 2. The contents of ginsenosides were the highest in $-Rg_1$ (19.96 mg/g), followed in decreasing order by $-Re$ (18.78 mg/g), $-Rd$ (9.83 mg/g), $-Rf$ (6.49 mg/g) and $-Rb_1$ (5.46 mg/g). This profile of saponins in ginseng leaves is notably different from that of saponins in ginseng roots,[14,15]; the ratio of protopanaxadiol to protopanaxatriol (PD/PT), that is proposed as a quality criterion in view of different physiolo-

Table 2. Ginsenoside composition of ginseng-leaf tea[a]

Content (mg/g, dry basis)								Total	Ratio[b] of PD/DT
Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1	Rg_2		
5.46 ± 0.24	3.51 ± 0.28	1.18 ± 0.12	9.83 ± 1.42	18.78 ± 1.84	6.49 ± 0.60	19.96 ± 2.02	1.68 ± 0.08	66.89	0.43

[a] Mean± standard deviation of triplicate determinations.
[b] Ratio of PD(panaxadiol ginsenoside $-Rb_1$, $-Rb_2$, $-Rc$, $-Rd$) to PT(Panaxatriol ginsenoside $-Re$, $-Rf$, $-Rg_1$, $-Rg_2$).

Table 3. Free sugar composition of ginseng-leaf tea[a]

Content (mg/g, dry basis)			Total
Fructose	Glucose	Sucrose	
8.82± 1.10	5.70± 0.42	6.25± 0.74	20.77

[a] Mean± standard deviation of triplicate determinations.

Table 4. Fatty acid composition of lipids from ginseng-leaf tea[a]

Fatty acids	Composition (%)
12 : 0	2.00
14 : 0	4.35
15 : 0	0.66
16 : 0	43.89
16 : 1	0.35
17 : 0	1.14
18 : 0	6.08
18 : 0	9.30
18 : 2	17.51
18 : 3	4.58
20 : 0	1.68
21 : 0	tr
22 : 0	3.39
23 : 0	1.52
24 : 0	3.55
TSFA[b]	68.26
TUFA[c]	31.74
PUFA[d]	22.09

[a] Each value is the mean of triplicate determinations.
[b] Total saturated fatty acids.
[c] Total unsaturated fatty acids.
[d] Polyunsaturated fatty acids (18 : 2+18 : 3).

gical activities of two fractions, is different between ginseng leaves (0.43) and ginseng roots (1.74).

These results for compositional properties of ginsenosides have confirmed previous reports,[14,31] proposing that ginseng leaves are a good source for ginsenoside $-Rg_1$, $-Re$ and $-Rd$. In particular, it was found from this work that ginseng-leaf tea maintained a high content of ginsenosides even under the manufacturing process and that this component could be an additional feature in the quality of ginseng-leaf tea over other leaf teas.

Free sugars of ginseng-leaf tea were composed of fructose (9.82 mg/g), sucrose (6.25 mg/g) and glucose (6.10 mg/g). This compositional profile of ginseng leaves was different from those of root and stem[38]; in ginseng root, sucrose is the highest, followed by glucose and fructose. In case of ginseng stem, however, the highest contents of sugars are observed in the order of glucose, sucrose and fructose. It is known that the sugtar content of white ginseng gradually decreases as its drying time increases.[39] The content (22.77 mg/g) of free sugars determined in this sample is similar to the result (21.10 mg/g) on raw ginseng leaves by Kim *et al.*,[38] and so there seems to be no apparent changes in free sugars under the manufacturing process of leaf tea.

Table 5. Amino acid composition of ginseng-leaf tea[a]

Amino acids	Content (mg/g, dry basis)	
	Total	Free
Aspartic acid	16.19	1.58
Threonine	6.46	0.11
Serine	7.50	0.33
Glutamic acid	20.73	0.47
Glycine	11.23	0.08
Alanine	8.03	0.23
Cystine	4.02	0.24
Valine	8.50	0.33
Methionine	1.26	0.01
Isoleucine	5.48	0.30
Leucine	11.12	0.32
Tyrosine	4.08	tr
Phenylalanine	8.63	0.36
Lysine	7.47	0.16
Histidine	2.69	0.05
Arginine	6.90	0.25
Proline	3.82	0.36
Total	134.10	5.18

[a] Each value is the mean of triplicate determinations.

Fatty acids: Fifteen fatty acids were analyzed by gas chromatography and percent composition in total lipids is given in Table 4. The major fatty acids in ginseng-leaf tea were palmitic (16 : 0, 43.89%), linoleic (18 : 2, 17.51%), oleic (18 : 1, 9.30%), and stearic acid (18 : 0, 6.08%). These findings were generally consistent with the results of raw ginseng leaves by Choi *et al.*[40] But some compositional diffeences wee observed showing a decrease in total

Table 6. Mineral composition of ginseng-leaf tea[a)]

Content (mg/g, dry basis)								
K	Na	Ca	Cu	Mg	Mn	Fe	Zn	P
18.50 ± 1.45	11.26 ± 1.00	28.72 ± 2.14	9.67 ± 0.82	4.04 ± 0.48	0.22 ± 0.06	2.68 ± 0.46	1.65 ± 0.36	1.82 ± 0.70

[a)] Mean ± standard deviation of triplicate determinations.

unsaturated fatty acid (TUFA) from 49.09% to 31.74 %, which might be resulted from heat treatment during the manufacturing process of leaf tea and/or from sample variations by age, 4 and 6 years old. It can be anticipated from the experiment that the steaming treatment possibly causes the changes in lipid constituents of ginseng leaves even though most results on thermal decomposition vary widely in the parameters of the heat treatment as well as in the analytical methods used for detection or measurement of the decomposition products.[41-43)]

Amino acuds: Seventeen selected amino acids were determined for ginseng-leaf tea and their contents are presented in Table 5. In total and free amino acid contents, glutamic acid, aspartic acid, valine, and leucine were relatively high in their contents but arginine content, which is normally high in ginseng roots, was low. This tendency was similar to that found in the amino acid compositions of ginseng-leaf protein concentrate and tea leaves.[19,32)] Amino acids, especially free amino acids are known to be closely related to the taste of tea leaves.[44)] Thus, considering the manufacturing process of ginseng-leaf tea, the steaming treatment possibly brings about chemical changes in the ginseng leaves.[44)]

Minerals: Very few reports are available on the effect of processing on mineal contents of ginseng products.[45)] Table 6 represents the mineral composition of ginseng-leaf tea. The major elements of the sample were found to be calcium, potassium, sodium, and copper, which are predominantly found in tea leaves.[46)] Similar results were reported by Mino[47)] on *Panax ginseng* leaves by X-ray fluorescence spectrometry, by Sung *et al.*[45)] on red ginseng, and by Kwon *et al.*[48)] on white ginseng powder. In a study on the relation between quality of tea and mineral composition, Kajita[46)] pointed out that the content of calcium and copper in tea could be a quality criterion and that they were found to be quite stable under the manufacturing process.

Based upon the aforementioned results, ginseng-leaf tea was found to contain higher amounts of soluble matter and ascorbic acid and lower contents of tannins, over green teas, showing a similar profile in their amino acid and mineral compositions.

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