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Quality Characteristics and Ginsenosides Composition of Ginseng-*Yakju* According to the Particle Size of Ginseng Powder

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ABSTRACT: The aim of this study was to develop rice wine (*Yakju*) containing various amounts and particle sizes of ginseng powder and to analyze the physicochemical characteristics and content of ginsenosides in ginseng-*Yakju*. Soluble solid content, pH, ethanol concentration, acidity, amino acid content, and evaluation of preference showed no difference between four kinds of *Yakju* groups, regardless of ginseng supplementation and particle size of the ginseng powder. During fermentation of *Yakju* containing ginseng, the contents of ginsenosides Rb1, Rb2, Rb3, and Rc were decreased. Otherwise, the content of ginsenoside Rh1 was increased highly by brewing microorganisms in *Yakju*. Recovery ratios of ginsenosides in ginseng-*Yakju* were approximately 25.4% (coarse ginseng power) and 23.8% (fine ginseng powder), which were superior to the recovery ratio of ginsenosides in *Yakju* containing ginseng slices (5%).

Keywords: finely pulverized ginseng, ginsenoside, ginsenoside Rh1, *Yakju*

INTRODUCTION

Increased incomes and rising interest in personal health have led to greater consumption of health-enhancing foods with specific physiological activities. Ginseng and red ginseng products are representative health-enhancing foods, and have physiological activities for anti-fatigue, anti-stress, improvement of immunity, and anti-carcinogenesis (1). Ginsenosides are saponins and the major pharmaceutical ingredient in ginseng. Approximately 30 different ginsenosides have been identified chemically and their pharmaceutical activities have been proved scientifically (2).

The compositions and amounts of ginsenosides are changed by processing procedures such as heating and hydrolysis. In our body, the ginsenosides are metabolized and modified chemically by intestinal microorganisms (1-4). Ginsenosides Rb1, Rb2, and Re are metabolized to ginsenosides Rd, Rg1, Rg2, Rh1, F1, and F2 by intestinal microorganisms, including *Bacteroides* sp., *Eubacterium* sp., *Fusobacterium* sp., and *Bifidobacterium* sp., and are converted to 20(S)-protopanaxadiol and protopanaxatriol (1). In addition, some intestinal lactic acid bacteria were involved in the metabolism of ginsenosides Rb1, Rb2, Rc, and Rd to compound K through ginsenoside F2 (5). However, the individual differences for intestinal microflora cause metabolic and physiological differences of ginsenosides in the body (1,6). Therefore, the bioactive and easily uptakable ginsenosides are necessary to be produced *in vitro* before eating, and possibly contribute to enhancing health.

Yakju, one of the traditional alcohol beverages in Korea, is a refined rice wine, filtering milky *Takju*. *Takju* is brewed by using rice and *Nuruk* as ingredients, which has a milky and turbid color due to the absence of clarification process after the fermentation. Starter *Nuruk* comes from the natural proliferation of wild microorganisms on a starch substrate of crushed or whole wheat or barley and have been used as a starter for alcohol fermentation (7). *Nuruk* contains various molds and yeasts, including mainly *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp., *Saccharomyces* sp., and *Pichia* sp. (8). As the interests for health-enhancing functions of *Yakju* increases, *Yakju* containing various fruits, berries, herbs, and medicinal plants has been developed.

In this study, we investigated the physicochemical characteristics of *Yakju* brewed using various shaped ginseng, and we studied the change of ginsenoside compositions and extraction yield in *Yakju*, according to the shape of added ginseng. This result is anticipated to provide fundamental information concerning the shape of added ginseng in the preparation of ginseng-*Yakju*.

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MATERIALS AND METHODS

Fermentation materials

Rice was harvested in Samcheok, Kangwon during October 2011. Korean ginseng was purchased from an agricultural cooperative's joint market in August 2011 in Samcheok, Kangwon, Korea. Water for fermentation was purchased from Dongwon F&B Co., Ltd. (Gyeonggi, Korea). Bionuruk®, which is a mixture of *Saccharomyces cerevisiae* and *Nuruk* microorganisms, was obtained from Korea Enzyme Inc. (Gyeonggi, Korea) and was used for *Yakju* fermentation as a fermentation starter. *Nuruk* is a starter containing a mixture of microorganisms, including *Aspergillus oryzae, Aspergillus niger*, and *Rhizopus oryzae*.

Drying and measuring free water content in ginseng

Ginseng was washed with water, and 0.5 cm slices of ginseng were dried with hot air in a sealed drier at 40°C for 72 h. Water content in ginseng was expressed as a weight after drying at 104°C for 16 h. Dry matter ratio was calculated by the following formula based on dry weight:

$$\text{Water content (\%)} = \frac{\text{Weight after dying}}{\text{Weight before drying}} \times 100$$

Preparation of ginseng powder

Dried ginseng slices were crushed mechanically by a pulverizer (Variable-Speed Rotor Mill Pulverisette 14, Fritsch GmbH, Idar-Oberstein, Germany). Working conditions were as follows: impact force; feed size, ~5 mm; sample quantity, 50 g; throughout, 10 g/min; sieve insert, 0.08, 0.2, 0.5, and 1.0 mm; feeding, batchwise process; rotor speed, 10,000 rpm; rotor type, 12 ribs (stainless steel). Crushed ginseng was isolated with 1.0 mm sieve insert, and coarse ginseng powders were grinded by a pulverizer equipped with 0.5, 0.2, and 0.08 mm sieve insert. Fine ginseng powder was defined as ginseng powder passed through size 0.08 mm sieve insert. In addition, some dried ginseng slices were not crushed and were used for preparation of *Yakju* (G1-*Yakju*) containing slices of ginseng. Size of ginseng powder was analyzed by a particle size analyzer (Analysette-22 NanoTec, Fritsch GmbH). Distribution ratio of particle diameter, average particle diameter, and specific surface area were measured in a range of 0.3~300 μm particle diameter.

Field emission scanning electron microscope (FE-SEM)

For observation of ginseng powder by FE-SEM, ginseng powder was coated with a gold plasma layer by ion sputter coater for 60 s in a vacuum condition. Microstructure of fine ginseng powder was observed with a FE-SEM at 5 kV.

Preparation of *Yakju* supplemented with ginseng powder

Rice (600 g) was washed and prepared for steamed rice. The hard-streamed rice, 120 g of Bionuruk® starter, and 60 g of yeasts were mixed in a 20 L of fermentation jar, and water was added to 20 L volume for basal *Yakju*. For a comparison, *Yakju* containing 0.2% of ginseng slices (G1-*Yakju*), *Yakju* containing 0.2% ginseng powder with 1.0 mm particle size (G2-*Yakju*), and *Yakju* containing 0.2% ginseng powder with 0.08 mm particle size (G3-*Yakju*) were used (Table 1). Material mixture for ginseng-*Yakju* was fermented in a fermentation jar equipped with air-lock apparatus for 7 days at 24°C, and was mixed completely twice a day. Samples (50 mL) were taken during fermentation and stored at −20°C for analysis. After a primary fermentation, a fermented mixture (*Yakju*) was stored at 4°C for 30 days for precipitation of the lees, and 10 L of *Yakju*, the supernatant layer, was isolated and used for analysis of ginsenoside and amino acids composition.

Analysis of total sugar content, pH, and acidity

Total sugar content of fermentation mixture was detected using 50 μL of filtered sample by a hand-held refractometer (model N-1α, ATAGO, Tokyo, Japan). The pH of fermentation mixture was measured by a pH meter (model 725p, Istek Inc., Seoul, Korea). Total titratable acidity (TTA) was analyzed by a titration method. Briefly, the fermentation mixture was spun by a centrifuge (5819R, Eppendorf, Hamburg, Germany) at 3,000 rpm and 4°C for 10 min, and the supernatant was filtered with a 0.45 μm syringe filter. One or two drops of phenolphthalein was added to a filtered sample (10 mL) and mixed with magnetic stirrer (model PC-420, Corning, Corning, NY, USA). NaOH (0.1 N) was added carefully to sample until color of sample became pink. End point of titration was defined as sample keeping a

Table 1. Composition of fermentation materials of ginseng-*Yakju*

	Yakju	G1-*Yakju*	G2-*Yakju*	G3-*Yakju*
Ginseng, sliced (g)	–	40	–	–
Ginseng, coarse powder (g)	–	–	40	–
Ginseng, fine powder (g)	–	–	–	40
Rice (g)	6,000	6,000	6,000	6,000
Water (mL)	13,580	13,540	13,540	13,540
Bionuruk® (g)	300	300	300	300
Yeast (g)	120	120	120	120

pink color for 30 s, and TTA was calculated from 0.1 N NaOH volume (mL) consumed to the end point using the following equation:

Total titratable acidity (TTA, %)=0.1 N NaOH (mL) ×factor of N NaOH×tartaric acid coefficient×100/ volume of sample (mL),

where tartaric acid coefficient is 0.075.

Analysis of ethanol content

Fermentation sample was centrifuged at 3,000 rpm and 4°C for 10 min, and the supernatant was filtered with a 0.45 μm syringe filter. Ethanol content in fermentation sample was quantitated by Alcolyzer (Anton Paar GmbH, Graz, Austria). Range for a confident concentration was 0～20% (v/v) and accuracy was approximately 0.1%.

Analysis of amino acids

For analysis of amino acid, a centrifuged sample was filtered with the 0.45 μm syringe filter and injected into the amino acid analyzer (L-8800, Hitachi, Tokyo, Japan) with ion exchange column (4.6 mm×60 mm). Column oven temperature, injection volume, flow rate, and reaction coil temperature were 30°C, 20 μL, 0.35 mL/min, and 135°C, respectively. Isolated amino acids from sample passed through the column were converted to colored compound by reaction with ninhydrin at high temperatures. Intensity of color was measured at 570 (for proline measurement) and 440 nm (for other amino acids) by the spectrophotometer. Content of free amino acids in sample was quantitated based on the intensity of color.

Analysis of ginsenosides

Ginseng powder (50 g) was added to 50 mL of methanol and extracted by ultrasonicator (JAC 2010, Jinwoo, Seoul, Korea) at 50°C for 1 h. Methanol extract was filtered with a 0.45 μm syringe filter, and 5 μL of filtered sample was analyzed by LC-MS (micromass ZQ detector, Waters, Milford, MA, USA) with a C18 column (3.5 μm, 2.1×150 mm, XTerra, Waters). In addition, *Yakju* sample was mixed with equal volume of methanol and filtered with a 0.45 μm syringe filter, and the supernatant was analyzed by HPLC. Mobile phases were created by a gradient solvent system using 18% acetonitrile (solvent A) and acetonitrile (solvent B). Gradient ratio of mobile phase was programed to hold at 100:0 (A : B, v/v) at 0～32 min, changed to 80:20 at 32～65 min, hold at 80:20 at 65～80 min, changed to 0:100 at 80～98 min, and kept at 0:100 (A : B, v/v) at 98～103 min at a 0.25 mL/min flow rate. Then, the gradient ratio of mobile phase was changed to 100:0 in 103～105 min and kept going to 110 min.

Sensory evaluation

Four different ginseng-*Yakju* samples (plain *Yakju*, G1-*Yakju*, G2-*Yakju*, and G3-*Yakju*) were brewed and stored for 18 h. A 19-student sensory evaluation panel from the Department of Food and Nutrition were pre-educated with purpose of study and characteristics of quality for ginseng-*Yakju*. Sensory evaluation was performed using a five-point scaling method (1=dislike extremely to 5=like extremely) for color, alcohol aroma, sweetness, bitterness, refreshing flavor, and viscosity of sample, acceptability, and preference using double blind test.

Statistical analysis

All results were analyzed by one-way analysis of variance (ANOVA) statistically (9), and were expressed as the mean±standard deviation.

RESULTS AND DISCUSSION

Drying of ginseng slices

After ginseng slices were dried in a sealed dryer at 40°C for 72 h, the weight change of the slices was measured, as shown in Table 2. After 24 h of drying, the weight of ginseng slices was reduced to approximately 30.7% of the control, which was the weight before drying ($P<0.001$). Then the ginseng slices were dried slowly and had approximately 27.4% of the control weight for 48-h drying. In addition, the ginseng had approximately 25.8% of the control weight for 16 h-drying at 104°C (data not shown). Generally, the process of food drying is composed of a settling down, a constant rate drying, and a falling rate drying period. In the case of drying ginseng, the falling rate drying period was reached within 24 h. In a subsequent study, we used 72 h-dried ginseng slices.

Table 2. Weight changes of sliced ginseng by drying process at 40°C

% Weight (g) of ginseng					*P*-value
0 h	12 h	24 h	48 h	72 h	
100±0[a1)]	57.0±4.2[b]	30.7±1.4[c]	27.4±0.7[c]	27.3±0.7[c]	0.001

[1)]Percentage (%) weight values are expressed as a ratio to the weight obtained at 0 h.
[a–c]Means with different superscripts are significantly different.

Table 3. Particle size distributions, mean diameters and specific surface areas of ginseng powder

Pulverizer insert sieve size (mm)	Diameter (μm) at:			Mean diameter (μm)	Specific surface area (cm^2/cm^3)	Supplementation to *Yakju*
	10%	50%	90%			
1	5.6	77.6	213.6	90.4	3,943	G2-*Yakju*
0.5	5.0	53.6	181.1	72.5	4,468	–
0.2	3.8	26.6	79.4	36.0	6,147	–
0.08	2.8	12.7	72.5	17.4	10,191	G3-*Yakju*

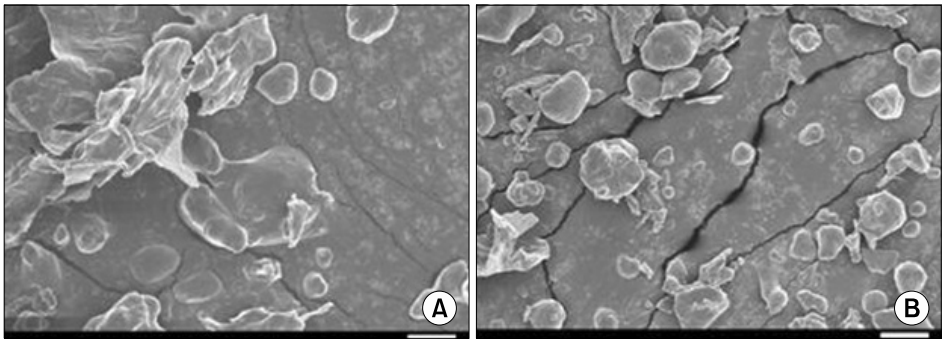

Fig. 1. Field emission-scanning electron microscopy of pulverized ginseng powder. (A) Pulverized ginseng powder passed through 1 mm sieve, (B) Pulverized ginseng powder passed through 4 sieves (1, 0.5, 0.2, and 0.08 mm). White bar indicates 10 μm.

Preparation of ginseng powder

As shown in Table 3, the ginseng powder was prepared to various particle sizes according to the mesh size (1.0, 0.5, 0.2, and 0.08 mm) of the pulverizer. Ginseng powder prepared by 1.0 mm sized sieve had particles approximately 5.6~213.6 μm diameters; the average particle size was 90.4 μm and the specific surface area was 3,943 cm^2/cm^3 (Table 3). The 0.5 and 0.2 mm sized sieves produced particle sizes approximately 5.0~181.1 and 3.8~79.4 μm in diameter, resulting in ginseng powder with average particle diameters of 72.5 and 36.0 μm and specific surface areas 4,468 and 6,147 cm^2/cm^3, respectively. Finally, a fine ginseng power with a 2.8~72.5 μm range was obtained by the 0.02 mm sized sieve with an average diameter of 17.5 μm and the specific surface area was 10,191 cm^2/cm^3. As the sieve size in the pulverizer was reduced, smaller ginseng particles and a wider specific surface area were obtained. In a milling process, rice, acorn, mung bean, and buckwheat have approximately 56.8~64.5, 12.1, 21.8, and 17.0 μm average particle size, respectively, which were similar to the particle size range of ginseng powder in this study (10,11).

In starch, the specific surface area affects various surface phenomena such as chemical reactions, catalysis, and absorption/desorption with different ingredients (12). Ginseng powder that was passed through the first sieve (1 mm sieve) was used for G2-*Yakju* fermentation, and a fine ginseng powder from the 0.08 mm sieve was used to brew G3-*Yakju*. The microstructures of the ginseng powder prepared by the 1 and 0.08 mm sieve were observed by a Field-Emission Scanning Electron Microscope (JSM-6701F/X-Max, JEOL Ltd., Tokyo, Japan) (Fig. 1). These two kinds of ginseng powder were mostly globular in shape and showed no distinct differences in appearance.

Physiochemical characteristics of *Yakju* containing ginseng

Rice wine *Yakju* was fermented using four different ingredient compositions (Table 1) in the particle size of ginseng powder for 7 days, and then was aged at 4°C for 30 days. Content of soluble solids (°Brix) was expressed as the concentration of sugars in fruits and liquids, and indicates the intensity of sweetness, an indirect measure of the degree of fermentation. In the early stage of fermentation for 2 days, the content of soluble solids was increased from 2.0~2.6°Brix to 9.4~10.0°Brix, and then did not change any further (Fig. 2A). Increase of soluble solids in ginseng-*Yakju* is attributed by a conversion of high molecular carbohydrates to low molecular products by an amylase of *nuruk* microorganisms. No differences in soluble solid content among the four groups were shown during fermentation period. Therefore, we assumed that the effect of supplemented ginseng powder on the content of soluble solids in *Yakju* is not great. In addition, the pH was decreased from 5.7~5.9 (on the first day) to 4.5 (at 1 day fermentation), and thereafter did not show a substantial change. At 7 days fermentation, the pH of all groups was approximately 4.3~4.4 (Fig. 2B), and no significant differences were observed among the test groups.

Ethanol contents were increased to 7.8~8.8 and 10.9~11.2% (v/v) at 1 day and 2 days fermentation, respectively, and no significant differences were observed among test groups. Additionally, the ethanol concentrations of ginseng-*Yakju* were approximately 13.3~13.7 at 7 days fermentation (Fig. 2B). Acidity is one of the

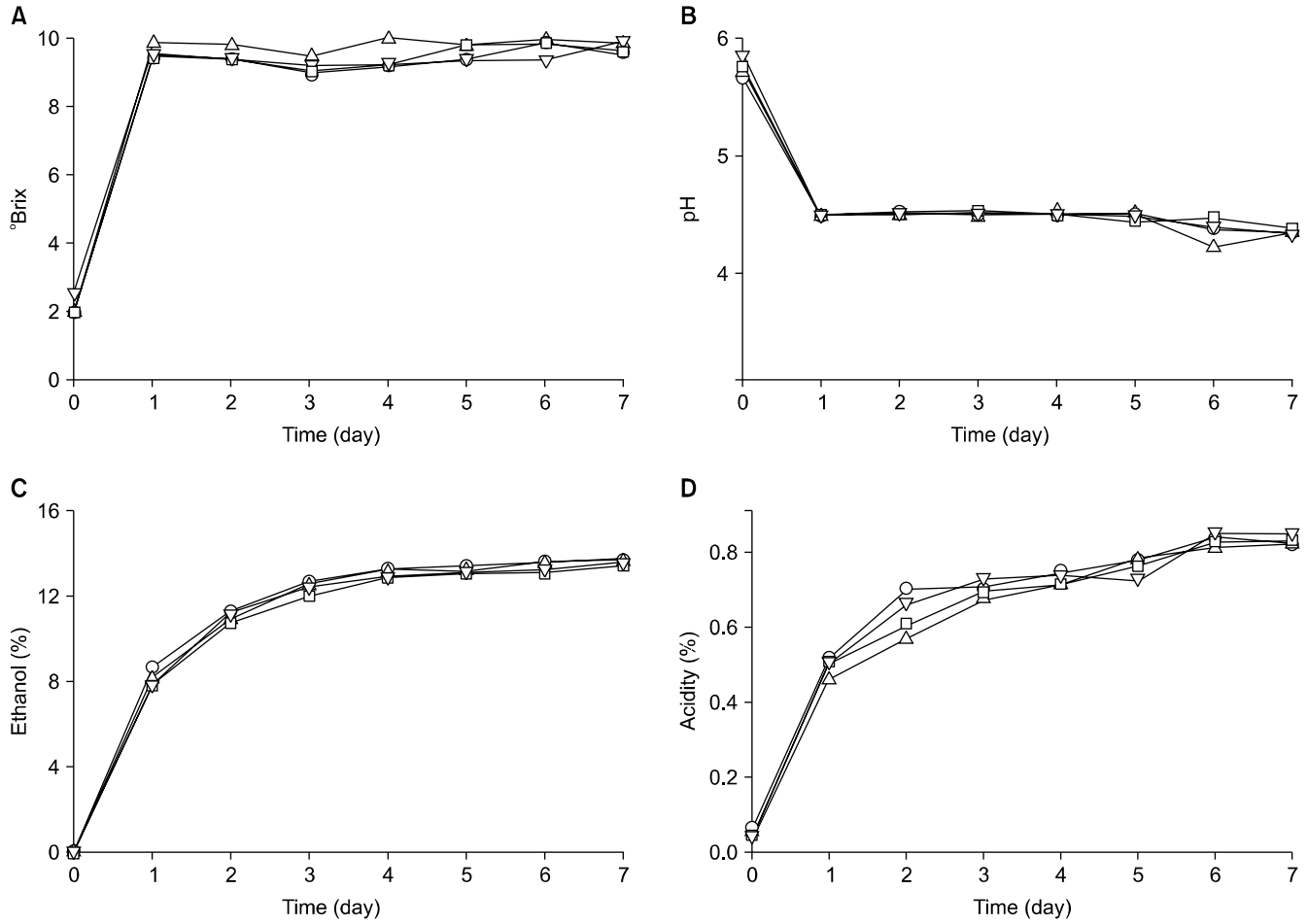

Fig. 2. Soluble solids content (°Brix) (A), pH (B), ethanol content (C) and total acidity (D) in ginseng-*Yakju* during fermentation at 24°C for 7 days. ○, *Yakju*; △, G1-*Yakju*; □, G2-*Yakju*; ▽, G3-*Yakju*.

factors that affected the sensory evaluation of ginseng-*Yakju*. Generally, *Yakju* with high sugar content and low acidity is strongly preferred to people. In this study, the acidity of ginseng-*Yakju* was increased from 0.03～0.04% as the fermentation is being processed. At 7 days fermentation, the acidities of the four test groups ginseng-*Yakju* were approximately 0.62～0.64% (Fig. 2D). Comparing *Yakju* with and without ginseng powder, the pH, ethanol contents, acidity, and soluble solid contents showed no significant differences and were consistent with a previous report (13).

Amino acid contents of ginseng-*Yakju*

Composition of amino acids affects the flavor and the taste of wines. In this study, the change of free amino acid composition was therefore determined in *Yakju* with and without ginseng powder. Free amino acid content was approximately 625～655 mg in 100 mL of *Yakju* and included glutamic acid (13.7～14.1%), arginine (11.0～11.7%), leucine (9.2～9.3%), and alanine (7.9～8.2%) (Table 4); the free amino acids composition was similar to the report described by Shon et al. (14) who reported that the major amino acids in steamed and raw rice were arginine, proline, leucine, arginine, tyrosine, and histidine. Generally, Korean ginseng and mountain ginseng contain approximately 680～1,250 mg/100 g of amino acids

Table 4. Amino acids of ginseng-*Yakju* after fermentation and aging

	Free amino acids (mg/100 g of *Yakju*)			
	Yakju	G1-*Yakju*	G2-*Yakju*	G3-*Yakju*
Aspartic acid	17	16	17	17
Threonine	24	22	23	24
Serine	38	36	37	38
Glutamic acid	90	87	92	90
Glycine	29	27	28	29
Alanine	52	51	52	52
Valine	38	37	38	38
Methionine	14	14	15	15
Isoleucine	25	24	24	25
Leucine	60	58	61	61
Tyrosine	41	40	40	42
Phenylalanine	44	43	44	45
Tryptophan	11	11	11	11
Lysine	44	43	47	46
Histidine	12	11	12	12
Arginine	73	73	74	72
Proline	32	32	39	38
Total	644	625	654	655

(15). Content of arginine is approximately 77.8% among the total amino acids of ginseng, and accounts for the highest content among 14 kinds of detected amino acids in ginseng (15). Thus, although the composition of amino acids in ginseng is distinguished from that of *Yakju*, the characteristics of ginseng amino acids did not affect the amino acid composition of *Yakju* from supplemented ginseng powder in this study, mainly due to the extremely smaller amount of ginseng (40 g) as compared with the amount of rice (6,000 g) in ginseng-*Yakju*.

Composition of ginsenosides in ginseng-*Yakju*

Approximately 30 ginsenosides have been identified to date. Among them, fourteen ginsenosides were analyzed in this study. Ginsenosides Rg1, Re, X, Rf, Rg2, Rh1, Rb1, Rc, Rb2, Rb3, Rd, Rg3, compound K, and Rh2 were detected in order, at 24.0, 25.9, 57.6, 60.0, 67.0, 68.3, 71.5, 74.3, 78.1, 79.6, 86.5, 93.2, 96.3, and 97.1 min of retention time, respectively, by measurement of LC-MS. In ginseng powder, the major ginsenosides were shown to be, in order, ginsenosides Re, Rb2, Rb1, Rc, Rg1, Rb3, and Rd. In G1-, G2-, and G3-*Yakju*, the major ginsenosides were Re and Rg1. In ginseng-*Yakju*, the ginsenosides Rb1, Rb2, Rb3, Rc, and Rd was not detected, but the main ginsenosides were Rf, Rg2, and Rh1 (Table 5). During brewing ginseng-*Yakju*, the kind and content of ginsenosides are supposed to be changed by chemical, physical, and microbial factors. To date, the metabolic pathway of ginsenosides by our body's intestinal microorganisms has been clearly described (1). However, the fermentation conditions of *Yakju*, which are comprehensively different from the circumstances of intestinal bioconversion, provide a specific metabolism of ginsenosides by *Yakju*-brewing microorganisms. *Nuruk*, a starter for brewing *Yakju*, is a mixture of microorganisms including bacteria, yeasts, and fungi. Supposedly, ginsenosides are metabolized by lactic acid bacteria, which are the major microorganisms in *Yakju*.

Table 5. Ginsenosides contents in ginseng-*Yakju* after fermentation and aging

	Content of ginsenoside (mg/kg)			
	Ginseng-powder	G1-*Yakju*	G2-*Yakju*	G3-*Yakju*
Rg1	2,080	0.063	1.90	1.87
Re	3,578	1.230	5.08	4.96
G-X	194	0.006	0.11	0.04
Rf	747	0.047	0.65	0.54
Rg2	373	0.131	0.38	0.38
Rh1	51	0.220	0.51	0.45
Rb1	2,763	0	0.11	0
Rc	2,134	0	0.03	0
Rb2	2,991	0	0	0
Rb3	975	0	0	0
Rd	962	0	0	0
Rg3	130	0.043	0	0
Compound K	320	0	0	0
Rh2	0	0	0	0
Total	17,282	1.740	8.77	8.24

Dammarane saponins in ginseng are classified to protopanaxadiol- and protopanaxatriol-saponins (2). Among them, ginsenosides Re and Rg1 are contained abundantly in ginseng (2). Ginsenoside Rh1, which was detected abundantly in ginseng-*Yakju* in this study, is known to have physiological activities for prevention of liver injury, stimulation for differentiation of cancer cells (F9 cells), inhibition of aggregation for platelets, and dissolution of platelets (2). Therefore, we need to study the physiological activity of *Yakju* containing ginseng powder in future studies.

Recovery ratios of ginsenosides in G1-, G2-, and G3-*Yakju* from supplemented ginseng were approximately 5.0, 25.4, and 23.8%, respectively. Twenty grams of ginseng powder has approximately 345.64 mg of ginsenosides. Ten liters of ginseng-*Yakju* were obtained from 20 L of ginseng-unrefined rice wine (*Makgeolli*). G1-, G2-, and G3-*Yakju* contained approximately 17.4, 87.7, and 82.4 mg/10 L of ginsenosides, respectively.

As compared to G1-*Yakju* using ginseng slices based on these results, G2- and G3-*Yakju* contained much more ginsenosides, which were extracted from ginseng powder. As shown in Table 5, G2-*Yakju* had more total ginsenosides contents than G3-*Yakju*. Though G3 ginseng powder had a larger total specific surface area than G2 ginseng powder (Table 3), the difference of ginseng particle sizes did not effective the amount of ginsenosides extracted in *Yakju*. Recovery yields of ginsenosides, which ware 5.0, 25.4, and 23.8% in G1-*Yakju*, G2-*Yakju*, and G3-*Yakju*, respectively, for 42 days fermentation/aging period, indicating the possibility that the pulverized ginseng powder may be precipitating at the bottom of the fermenter. Therefore, the sufficient agitation during fermentation and aging period as well as the refining of ginseng is expected to greatly boost recovery yields of ginsenosides.

Sensory evaluation of ginseng-*Yakju*

A sensory evaluation for four groups of lab-brewed *Yakju* with/without ginseng and with different particle sizes of ginseng powder was performed using a 5-point scaling method (from 1=dislike extremely to 5=like extremely) for color, alcohol aroma, sweetness, sour taste, bitterness, refreshing flavor, and viscosity in a double-blind test, a profiling method, and a preference evaluation (Table 6). In all test groups, a sensory evaluation for color, alcohol aroma, sweetness, sour taste, bitterness, refreshing flavor, and viscosity section were scored as approximately 3.1～3.8, 3.2～3.4, 2.0～2.3, 3.0～3.2, 2.9～3.4, 2.8～3.1, and 2.2～2.3 points, respectively. In the color section, G1-, G2-, and G3-*Yakju* were evaluated highly at a sig-

Table 6. Sensory characteristics and preference responses of ginseng-*Yakju*

	Yakju	G1-*Yakju*	G2-*Yakju*	G3-*Yakju*	Significance
Sensory characteristics					
Color	3.1±0.6[b]	3.8±0.6[a]	3.6±0.6[ab]	3.8±0.7[a]	$P<0.05$
Alcohol aroma	3.3±0.7	3.4±1.0	3.3±0.8	3.2±0.8	NS[1)]
Sweetness	2.2±0.9	2.0±0.7	2.3±0.9	2.3±0.7	NS
Sour taste	3.0±1.1	3.0±1.2	3.2±1.0	3.0±0.9	NS
Bitterness	3.4±1.0	2.9±1.2	3.4±0.9	3.3±0.9	NS
Refreshing flavor	3.1±0.7	2.9±0.9	3.0±0.8	2.8±0.7	NS
Viscosity	2.2±0.9	2.2±0.8	2.3±0.9	2.3±0.8	NS
Preferences responses to the sensory characteristics					
Color	3.1±0.6	3.4±0.8	3.4±0.8	3.3±0.8	NS
Alcohol aroma	2.8±0.9	2.7±0.7	3.0±0.9	2.9±0.7	NS
Sweetness	2.5±0.9	2.3±0.9	2.3±0.7	2.4±0.8	NS
Sour taste	2.8±0.9	2.7±0.9	2.8±0.9	2.9±0.7	NS
Refreshing flavor	2.9±0.6	2.9±0.8	3.1±0.8	2.9±0.6	NS
Viscosity	3.4±0.5	3.5±0.5	3.6±0.6	3.5±0.5	NS
Overall acceptability	2.7±0.8	2.7±0.9	2.9±0.8	3.0±0.8	NS

[1)]NS, not significant.
[a,b]Means with different superscripts are significantly different.

nificant difference ($P<0.05$) as compared with plain *Yakju*. According to the report concerning fermented ginseng-*Yakju* by Ahn and Lee (16), the fermented ginseng-*Yakju* had light yellow materials with high peaks at 232 and 258 nm by spectrophotometry. Color of fermented ginseng-*Yakju* in this study appeared a strong yellow compared to normal *Yakju*. However, in all other sections, no differences were found among the four test groups. In the preference evaluation, the color, alcohol aroma, sweetness, sour taste, bitterness, refreshing flavor, viscosity, and overall acceptability section were scored approximately as 3.1～3.4, 2.7～3.0, 2.3～2.5, 2.7～2.9, 2.6～2.8, 2.9～3.1, 3.4～3.6, and 2.7～3.0 points, respectively. Although the preference among four groups showed no significant differences with each other, G2- and G3-*Yakju* were evaluated highly, as compared to plain *Yakju*, in terms of overall acceptability. This result indicates that the supplementation of ginseng does not adversely affect the original sensory characteristics of *Yakju*. However, other researchers have reported that the excessive supplementation of ginseng to rice wine had a negative effect on flavor and taste of rice wines (16). The less supplement of ginseng was the better sensory evaluation in fermented ginseng-*Yakju*. Addition of ginseng (0.13～0.4%) showed a similar flavor but adding more than 2% ginseng reduced the sensory evaluation of fermented ginseng-*Yakju* (16). Therefore, considering the characteristics of flavor and price-aspect, the 0.2% ginseng was supposedly appropriate for brewing ginseng-*Yakju* in this study.

In this study, we fermented *Yakju* containing ginseng with different amounts and particle size, and we investigated the physiochemical characteristics and contents of functional ginsenosides in ginseng-*Yakju*. Particle size of the ginseng powder was approximately 17.4 and 90.4 μm depending on the sieve size of the pulverizer. In the fermentation of *Yakju* using ginseng (40 g) and rice (6,000 g), a plain *Yakju*, *Yakju* containing ginseng slices (G1-*Yakju*), *Yakju* containing a rough powder of ginseng (G2-*Yakju*), and *Yakju* containing a fine powder of ginseng (G3-*Yakju*) had similar characteristics regarding soluble solids, pH, ethanol concentration, acidity, amino acid content, and sensory evaluation. Major ginsenosides of the ginseng powder were Re, Rb2, Rb1, Rc, Rg1, Rb3, and Rd, and the total content of ginsenosides was approximately 1.7 g/100 g of dried ginseng powder. During fermentation of *Yakju* containing ginseng, the content of ginsenosides Rb1, Rb2, Rb3, and Rc was decreased, but the amount of ginsenoside Rh1 was increased highly. Change of ginsenoside composition during *Yakju* fermentation is believed to be caused by metabolism of ginsenosides by brewing microorganisms in *Yakju*. We speculated that the physiological functionality of *Yakju* containing ginseng might be altered by fermentation. Based on these results, we can conclude that the shape of added ginseng in the preparation of ginseng-*Yakju* is responsible for the extraction of ginsenosides in *Yakju*. Additionally, these results suggest that the preparation of *Yakju* using pulverized ginseng has a higher bioavailability of ginsenosides than normal fermented ginseng-*Yakju*.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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