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# Quality Characteristics and Biological Activities of Traditionally Fermented Ginseng Wine

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**Abstract** The objectives of this study were to determine the quality characteristics, antioxidant activity, and cytotoxicity of fermented ginseng wine at each fermentation step. In the first mash with and without ginseng, viable cell counts (total cell, lactic acid bacteria, and yeast) were maximum between 2 to 4 days of fermentation. At the beginning of fermentation, Brix and ethanol contents, and titratable acidity increased, while pH decreased rapidly. At 3 days of fermentation of the second mash with ginseng, the viable cell counts were similar to those without ginseng and then continually decreased. At the end of fermentation, the pH of the second mash with ginseng was 4.00, lower than the pH of the second mash without ginseng, which was 4.35. Alcohol contents of second mashes with and without ginseng were 12.2 and 11.8%, respectively. In the aging period of ginseng and rice wines, the pH, titratable acidity, Brix, and ethanol contents did not change markedly. The results of sensory evaluation showed that fermented ginseng wine had good flavor and high acceptability. In the 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity assay, fermented ginseng wine ($IC_{50}$: 0.394 mg/mL) showed higher antioxidant activity than fermented rice wine ($IC_{50}$: 0.884 mg/mL). The butanol fraction of fermented ginseng wine exhibited weak cytotoxic activity against P388 and HeLa cell lines.

**Keywords:** antioxidant activity, cytotoxicity, fermented ginseng wine, viable cell count, sensory evaluation

## Introduction

The main ingredients of Korean traditional rice wines are steamed rice, *nuruk* (Korean-style mold bran) and water. *Nuruk* has been used traditionally as a wine starter. *Nuruk*s are made from various grains, but *nuruk* from whole wheat grits is used predominantly (1). To manufacture *nuruk*, whole wheat grits are moistened, pressed into cakes and then the cakes are fermented naturally allowing them to develop natural microflora (mold, bacteria, and yeast) (1, 2). Jo and Ha (3) identified 32 strains of lactic acid bacteria from *nuruk* and reported *Leuconostoc mesenteroides* as the predominant one. Fermentation may increase the functionality of food and improve its organoleptic properties due to the acidic taste and the unique flavors that develop during fermentation (4).

Medicinal herbs (*sesame, gilkyung, jakyak, danggwi, hwangki,* and *chunkung*) were added to the distillate of Korean rice wine and the quality of the resulting medicinal herb liquors examined (5). Yu *et al.* (6) mentioned that the addition of medicinal plants or mushrooms into the mash increased the physiological functionality of traditional Korean rice wine. Korean traditional rice wine with medicinal herbs can be prepared by two different methods; fermenting rice wine with medicinal herbs or soaking medicinal herbs in distilled liquor (1, 5, 7, 8). Ginseng, which contains a protopanaxadiol group (ginsenosides Ra, Rb1, Rb2, Rc, and Rd) and a protopanaxatriol group (ginsenosides Rg1, Re, Rf, and Rg2), has been used as a traditional medicine in Korea, China, and Japan (9, 10). It has been reported that ginseng has anticancer, antihypertension, antidiabetes, and antistress effects and can facilitate learning (11-13).

The objectives of this study were to determine the quality characteristics of ginseng wine during fermentation and the aging period and to determine the antioxidant activity and cytotoxicity of each fermentation step.

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## Materials and Methods

**Preparation of fermented ginseng wine** Fresh ginseng and wheat *nuruk* were purchased from Gyeongdong Market. Fresh ginseng was washed, drained, dried at 60°C for 2 days and powdered by mill (Sample Mill SK-M 10R; Kyoritsu Riko Co., Tokyo, Japan). The ingredients used in the preparation of fermented ginseng and rice wines are shown in Table 1. For the first mash of ginseng wine, 420 g of nonglutinous rice (Pyeongtaek, Gyeonggi, Korea) was soaked for 12 hr at room temperature (20°C) and then cooked for 40 min on the gas range using a steamer. The cooked rice was spread on the pan and cooled to room temperature. Thirty g of ginseng powder, 250 g of *nuruk*, and 1 L of cold water were added to the cooked rice, and then mixed thoroughly to a paste. This first mash was fermented at 24°C for 3 days. For preparation of the second mash, 2.42 kg of glutinous rice (Icheon, Gyeonggi, Korea) was soaked for 12 hr, cooked and cooled. Eighty g of ginseng powder, 1.4 L of cold water, and the first mash were added to the cooked rice, and then mixed thoroughly and fermented at 20°C for 15 days. Rice wine was prepared by the same method as ginseng wine, but without the addition of ginseng powder. After fermentation of the second mash, it was filtered through a 20 mesh sieve and the filtrate was aged at 15°C for 15 days.

**Viable cell counts** The viable cell counts (total cell,

lactic acid bacteria, and yeast counts) were analyzed during the fermentation period of the first and second mashes, and during the aging period of the ginseng and rice wines. Standard method agar was used for the total cell count, lactobacilli MRS agar for the lactic acid bacteria count, and Sabouraud dextrose agar:Bacto agar (2: 1) for the yeast count.

**pH and titratable activity** The ginseng and rice wines during fermentation period were analyzed for pH and titratable acidity. Ten mL ginseng wine and 100 mL deionized water were mixed and then the pH was determined. Ten mL ginseng wine and 10 mL deionized water were mixed and 2-3 drops of phenolphtalein were added. This mixture was then titrated to an end point of pH 8.1 with 0.1 N NaOH. The acidity of ginseng wine was calculated as % lactic acid.

**Brix** The Brix values of ginseng and rice wines during the fermentation period were measured by refractometer (N-1 α; ATAGO, Tokyo, Japan).

**Alcohol content** Fifty mL samples of ginseng or rice wine were taken during the fermentation period. Fifty mL deionized water was added, the wine mixed, and then distilled to obtain 70 mL distillate. Thirty mL deionized water was added to this distillate and then the alcohol content was determined using an alcoholmeter (Daekwang Co., Seoul, Korea). The alcohol content of the wine was calculated by multiplying alcoholmeter reading by two.

**Table 1. Ingredients of fermented ginseng wine**

| | Ingredient | Fermented rice wine | Fermented ginseng wine |
|---|---|---|---|
| First mash | Nonglutinous rice (g) | 450 | 420 |
| | Ginseng powder (g) | - | 30 |
| | *Nuruk* (g) | 250 | 250 |
| | Water (mL) | 1000 | 1000 |
| Second mash | Glutinous rice (g) | 2500 | 2420 |
| | Ginseng powder (g) | - | 80 |
| | Water (mL) | 1400 | 1400 |

**Sensory evaluation** After the aging period was finished, ginseng and rice wines were filtered and stored in a refrigerator for 24 hr. These wines were evaluated for their sensory quality by a 21-student panel of the university. Seven-point hedonic scales (1 = dislike extremely, 7 = like extremely) for color, taste, flavor, and overall acceptability were used to evaluate the wines.

**Antioxidative effect** 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of ginseng and rice wines at each fermentation step were measured by the method of Xiong *et al.* (14).

***In vitro* cytotoxicity assay** *In vitro* cytotoxicity of the butanol fractions of ginseng and rice wines were evaluated against P388 (mouse lymphoid neoplasma), HeLa (human cervix adenocarcinoma), and A549 (human lung carcinoma) cell lines by 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the method of Carmichael *et al.* (15). Each cultured cell line was harvested, counted, and inoculated at the appropriate concentrations (180 μL volume: $4\times10^4$ cells/well for P388; $3\times10^4$ cells/well for HeLa and A549) into 96-well microtiter plates. The P338 cell line was then cultured for 2 hr, whereas the HeLa and A549 cell lines were cultured for 24 hr. The cells were exposed to the test compounds at 37°C for 2 days. Fifty μL of MTT solution (2 mg/mL in phosphate buffered saline) was added to each well and the plates were incubated for 4 hr. After aspiration of the medium, dimethyl sulfoxide (100 μL) was added to solubilize the MTT-formazan product. The absorbance was measured by enzyme-linked immunosorbent asaay reader at 540 nm. The 50% inhibitory concentration ($IC_{50}$) of tumor cell growth was defined using the various cells cultured without the test compounds as the controls.

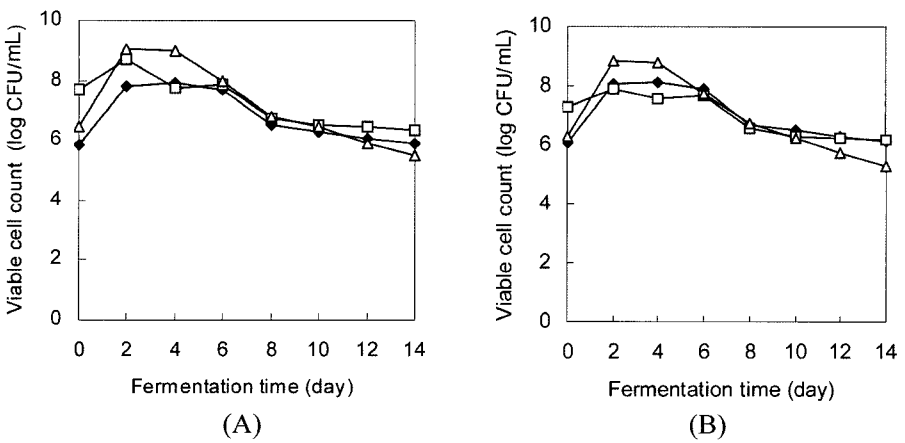


**Fig. 1. Changes in viable cell counts of the first mash with (A) and without ginseng (B) during 14 days of fermentation at 24°C.** ◆, total viable cells; □, lactic acid bacteria; △, yeast.

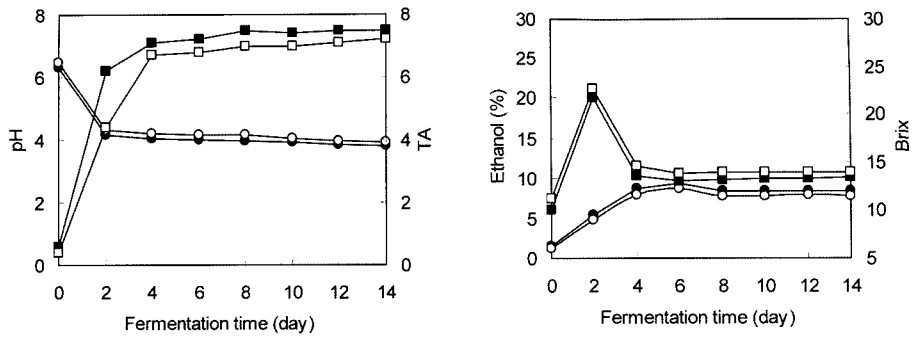


**Fig. 2. Changes in pH and titratable acidity (TA) (A) and Brix and ethanol content (B) of the first mash during 14 days of fermentation at 24°C.** ●, ginseng wine (pH, ethanol); ○, rice wine (pH, ethanol); ■, ginseng wine (TA, Brix); □, rice wine (TA, Brix).

## Results and Discussion

**Characteristics of the first mash fermentation of ginseng wine** The viable cell counts (total cell, lactic acid bacteria, and yeast counts) of the first mashes with and without ginseng during 14 days of fermentation at 24°C are shown in Fig. 1. From 2 to 4 days of the first mash fermentation period, total cell, lactic acid bacteria, and yeast counts were maximal. In the first mash with ginseng, total cell count was 7.90 log CFU/mL, lactic acid bacteria count was 7.78 log CFU/mL, and yeast count was 8.98 log CFU/mL at 4 days of fermentation. In the first mash without ginseng, the total cell count was 8.10 log CFU/mL, lactic acid bacteria count was 7.58 log CFU/mL and yeast count was 8.78 log CFU/mL at 4 days of fermentation. Between 4 to 14 days of fermentation, viable cell counts (total cell, lactic acid bacteria, and yeast) decreased continuously. So (16) reported that lactic acid bacteria in the first mash of *sogukju* were the most predominant microflora after 3 days of fermentation.

The pH and titratable acidity of the first mashes with and without ginseng during 14 days of fermentation at 24°C are shown in Fig. 2. During the first 2 days of fermentation of the first mash, the pH decreased rapidly and then decreased gradually. The pH of the first mash with ginseng decreased from 6.30 at day 0 to 3.79 after 14 days of fermentation. The pH of the first mash without ginseng decreased from 6.48 at day 0 to 3.91 after 14 days. There was a trend for the pH to be lowered in the first mash with ginseng throughout the fermentation period. Kim (17) reported that the butanol and aqueous layers of ginseng extract lowered the pH of the medium after culturing with lactic acid bacteria. As shown in Fig. 1, at 2 days of fermentation, the first mash with ginseng (8.72 log CFU/mL) had a higher number of lactic acid bacteria than that without ginseng (7.90 log CFU/mL).

The Brix and ethanol contents of the first mashes during 14 days of fermentation at 24°C are also shown in Fig. 2. The Brix was the highest after 2 days of fermentation of the first mash. The Brix of the first mash with ginseng increased from 10.0 °Bx at day 0 to 21.7 °Bx at 2 days of fermentation. The Brix of the first mash without ginseng increased from 11.2 °Bx at day 0 to 22.6 °Bx at 2 days. These increases were due to the hydrolysis of starch by amylase in the *nuruk*. After 2 days of fermentation of the first mash with and without ginseng, Brix decreased rapidly and then reached a plateau which was maintained. The ethanol contents of the first mash with and without ginseng increased rapidly from 1.5 and 1.2% at day 0 to 8.7 and 8.0% at 4 days of fermentation, respectively, due to the high amount of yeast. In the first mash with and without ginseng, yeast counts were 8.98 and 8.78 log CFU/mL at 4 days of fermentation. The yeast count then decreased continuously (Fig. 1).

In traditional rice wine preparation, the first mash is made in order to increase the number of lactic acid bacteria and yeast. By adding the first mash to the second mash, fermentation of the second mash is facilitated by the high number of yeast in the first mash and the growth of unfavorable bacteria in the second mash fermentation are prevented by acid produced from the lactic acid bacteria (1). Park *et al.* (4) noted that lactic acid generated in fermented food can inhibit the growth of putrefactive bacteria and maintain the food quality during storage. Therefore, day 3 of fermentation of the first mash is the optimum time to add the second mash preparation.

**Characteristics of the second mash fermentation of ginseng wine** The viable cell counts (total cells, lactic acid bacteria, and yeast counts) of the second mash with and with ginseng during 15 days of fermentation at 20°C are shown in Fig. 3. At day 0 of fermentation of the second mash, total cell, lactic acid bacteria, and yeast counts were higher than at 3 days of fermentation of the first mash in Fig 1. This was due to the incorporation of viable cells from the ginseng, rice, and water in the mixing step. On day 3 of fermentation of the second mash with ginseng, the total cell count was 8.13 log CFU/mL, lactic acid bacteria count was 8.82 log CFU/mL and yeast count was 8.91 log CFU/mL. The viable cell counts of the second mash without ginseng on day 3 were similar to those with ginseng. On day 6 of fermentation, the second mash with ginseng had a higher number of lactic acid bacteria and yeast (8.11 and 8.40 log CFU/mL, respectively) than the second mash without ginseng (7.45 and 7.00 log CFU/mL, respectively). However, after 9 days of fermentation of the second mash, total cell, lactic acid bacteria, and yeast counts with and without ginseng continually decreased, because the increasing alcohol production by yeast inhibited viable cell growth.

The pH and titratable acidity of the second mash during 15 days of fermentation at 20°C are shown in Fig. 4. The pHs of the second mash with and without ginseng were 4.72 and 4.96 at day 0 of fermentation. After 15 days of fermentation, the pH of the second mash with ginseng was 4.00, which was lower than the pH of the second mash without ginseng, which was 4.35.

The Brix and ethanol contents of the second mash during 15 days of fermentation at 20°C are also shown in Fig. 4. The Brix of the second mash with and without ginseng were 25.1 and 27.0 °Bx respectively at day 0 of fermentation. During 15 days of fermentation of the second mash, the Brix was maintained at a plateau by the ongoing hydrolysis of starch balanced by the continuing alcohol production by yeast. The alcohol content of the second mash with ginseng increased from 1.2% at day 0 to 7.9% on day 2 of fermentation. The alcohol content of the second mash without ginseng increased from 1.4% at day 0 to 7.3% day 2 of fermentation. After 3 days of fermentation of the second mash with and without ginseng, the alcohol content increased slowly. At the end of the fermentation period, the alcohol contents of the second mash with and without ginseng were 12.2 and 11.8% respectively. The growth of lactic acid bacteria isolated from *nuruk* has been shown to be inhibited at 10% ethanol and a pH of 4.0 (18). At 9 days of fermentation, the pH of the second mash with and without ginseng decreased to 3.95 and 4.17, and ethanol contents increased to 10.0 and 9.2%. The number of lactic acid bacteria in the second mash with ginseng rapidly dropped from 8.11 log CFU/mL at 6 days to 5.85 log CFU/mL at 9 days of fermentation.

**Characteristics of ginseng wine during aging** After 15 days of fermentation, the second mashes were filtered by a 20 mesh size sieve and the filtrates were aged at 15°C for 15 days. The viable cell counts (total cell, lactic acid bacteria, and yeast counts) of ginseng and rice wines during 15 days of aging at 15°C are shown in Fig. 5. During the first 3 days of aging, there was a slight tendency for total cell, lactic acid bacteria, and yeast counts to increase in both ginseng and rice wines. On day 3 of aging of ginseng wine, total cell count was 6.66 log CFU/mL, lactic acid bacteria count was 6.45 log CFU/mL, and yeast count was 6.53 log CFU/mL. However, after these first 3 days, the viable cell counts gradually decreased. Compared to viable cell counts in the second mash during the fermentation period, the number of viable cells in both ginseng and rice wines during the aging period were low.

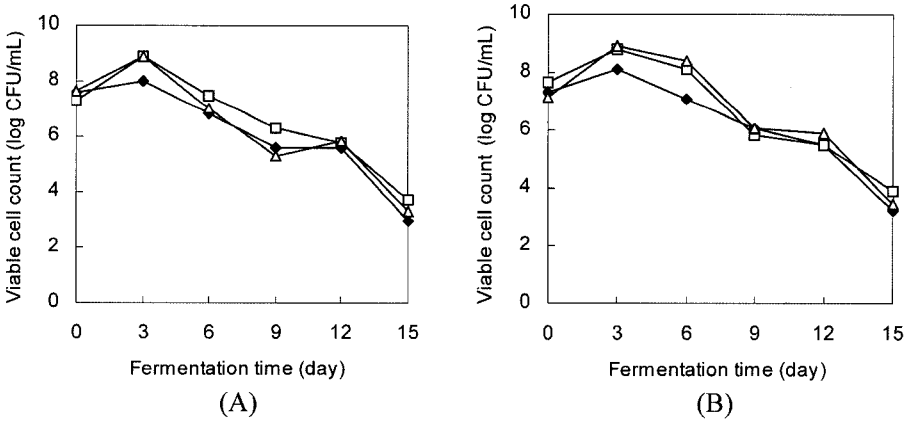


**Fig. 3. Changes in viable cell of the second mash with (A) and without ginseng (B) during 15 days of fermentation at 20°C.** ◆, total viable cells; □, lactic acid bacteria; △, yeast.

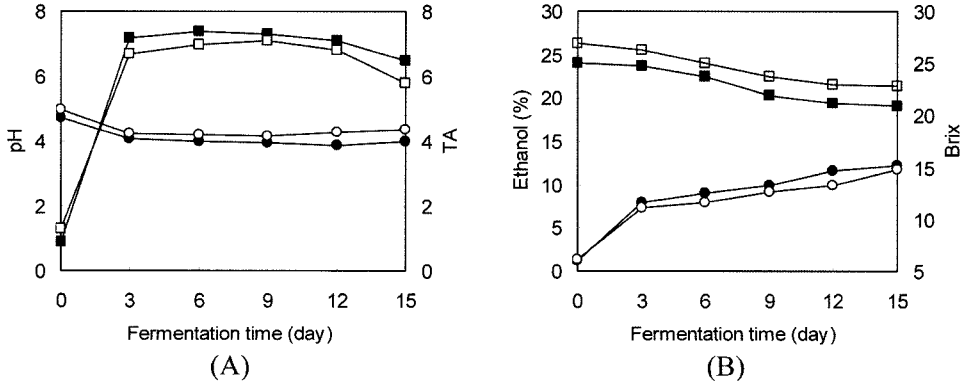


**Fig. 4. Changes in pH and titratable acidity (TA) (A) and Brix and ethanol content (B) of the second mash during 15 days of fermentation at 20°C.** ●, ginseng wine (pH, ethanol); ○, rice wine (pH, ethanol); ■, ginseng wine (TA, Brix); □, rice wine (TA, Brix).

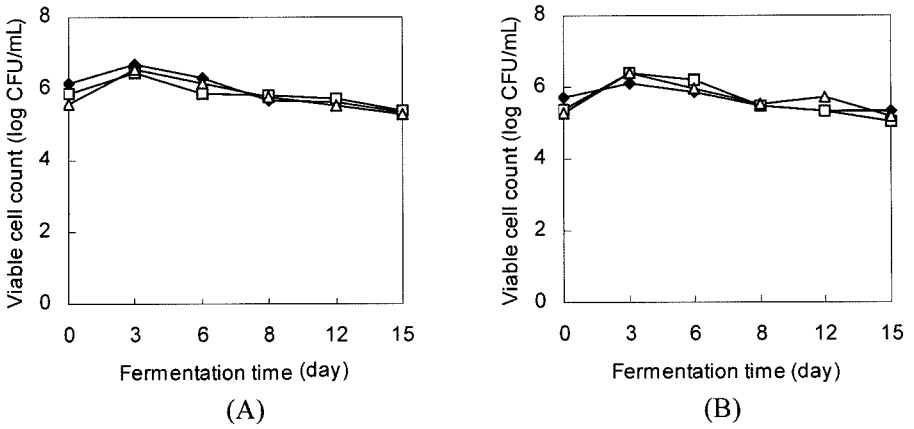


**Fig. 5. Changes in viable cell counts of ginseng wine (A) and rice wine (B) during 15 days of aging at 15°C.** ◆, total viable cells; □, lactic acid bacteria; △, yeast.

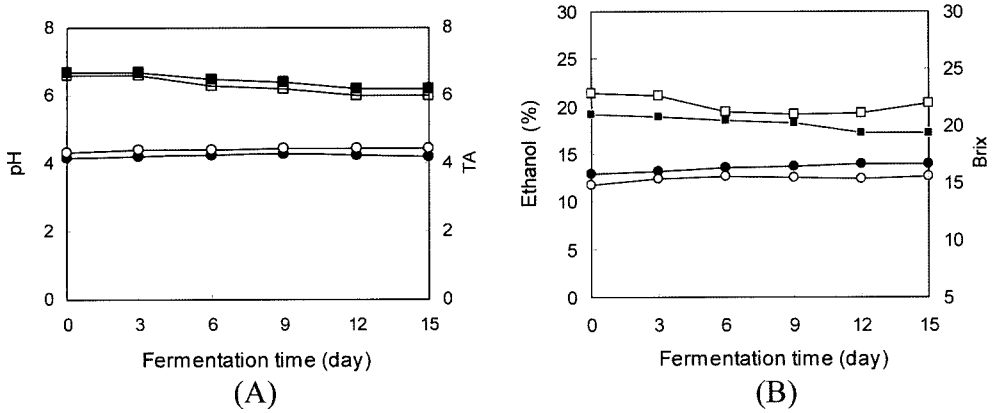


**Fig. 6. Changes in pH and titratable acidity (TA) (A) and Brix and ethanol (B) of ginseng and rice wines during 15 days of aging at 15°C.** ●, ginseng wine (pH, ethanol); ○, rice wine (pH, ethanol); ■, ginseng wine (TA, Brix); □, rice wine (TA, Brix).

The pH and titratable acidity of ginseng and rice wines during 15 days of aging at 15°C are shown in Fig. 6. During the aging period, the pH and titratable acidity maintained a stead plateau in both ginseng and rice wines. The pHs of ginseng and rice wines were 4.15 and 4.32 at day 0 of aging. After 15 days of aging, the pHs of ginseng and rice wines were 4.20 and 4.46, and the titratable acidity of ginseng and rice wines were 6.2 and 6.0%.

The Brix and ethanol contents of ginseng and rice wines during 15 days of aging at 15°C are also shown in Fig. 6. The Brix of ginseng and rice wines were 21.0 and 22.8 °Bx at day 0 of aging. During 15 days of aging, the Brix maintained a steady plateau. The alcohol contents of ginseng and rice wines were 12.9 and 11.8%, respectively at day 0 of aging. After 15 days of aging, the alcohol content of ginseng wine was 14%, higher than that of rice wine, which was 12.6%. Yeasts cannot tolerate the alcohol that they produce beyond levels of around 12-15% alcohol by volume. Therefore natural wines generally contain 9-13% alcohol from fermentation (19).

**Sensory evaluation of ginseng wine** The mean sensory scores of various wines are given in Table 2. Scores for color, taste, flavor, and overall acceptability were significantly different between wines. Color scores of fermented ginseng wine (5.71) and commercial ginseng wine (5.57) were better those of fermented rice wine (4.57) and commercial *cheongju* (4.95). Commercial ginseng wine and fermented ginseng wine received higher taste scores (5.14 and 4.95 respectively) than did fermented rice wine (3.67). There was no significant difference in taste score between fermented rice wine and commercial *cheongju*. Fermented ginseng wine had a more desirable flavor (6.90) than commercial ginseng wine (4.71). Since commercial ginseng wine is prepared by distillation, it may lack the flavor produced by fermentation. In the overall acceptability score, fermented ginseng wine and commercial ginseng wine scored higher (5.48 and 4.95 respectively) than fermented rice wine and commercial *cheongju* (3.62 and 4.05 respectively). During fermentation of rice wine, yeast and lactic acid bacteria in *nuruk* may produce various flavoring compounds as well as alcohol and acid. The results of the sensory evaluation showed that fermented ginseng wine had a good flavor and high acceptability.

**Biological activities** Antioxidant activities of ginseng and rice wines in each fermentation step were analyzed by measuring DPPH radical scavenging activities. The inhibitory effects of ginseng and rice wines in each

**Table 2. Sensory evaluation of various wines**

| | Sensory score[1)] | | | |
|---|---|---|---|---|
| | Color | Taste | Flavor | Overall acceptability |
| Fermented ginseng wine | 5.71±1.35[a2)] | 4.95±1.60[a,b] | 6.00±1.26[a] | 5.48±1.47[a] |
| Commercial ginseng wine | 5.57±0.98[a] | 5.14±1.39[a] | 4.71±1.45[b] | 4.95±1.28[a] |
| Fermented rice wine | 4.57±1.12[b] | 3.67±1.74[c] | 3.95±1.40[b] | 3.62±1.36[b] |
| Commercial *cheongju* | 4.05±1.50[b] | 4.00±1.55[b,c] | 4.05±1.50[b] | 4.05±1.53[b] |
| F-value | 8.59 ($p$<0.0001) | 4.37 ($p$=0.0066) | 9.46 ($p$<0.0001) | 7.47 ($p$<0.0002) |

[1)]1 = dislike extremely, 7 = like extremely.
[2)]Mean values in each column followed by different superscripts are significantly different at the $p$<0.05 level.

fermentation step on the DPPH radical scavenging activity are shown in Table 3. The $IC_{50}$ value of fermented ginseng wine (0.394 mg/mL) was lower than that of fermented rice wine (0.884 mg/mL). The antioxidant activity increased during fermentation of ginseng wine. The first mash with ginseng had lower antioxidant activity than the second mash with ginseng and fermented ginseng wine. Also, the first and second mashes without ginseng and fermented rice wine had low antioxidant activity.

Ginseng extract has been shown to be a scavenger of DPPH and superoxide radicals (20, 21). Kim *et al.* (21) reported that 2 mg/mL ginseng extract completely eliminated DPPH and superoxide radicals. The production of oxygen-derived free radicals might be related to many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as aging (22, 23). Therefore, moderate consumption of fermented ginseng wine might prevent these diseases.

The cytotoxicities of the butanol fractions of ginseng and rice wines in each fermentation step against P388, HeLa, and A549 cells were analyzed (Table 3). After fermentation of ginseng wine, cytotoxic activities against P388 and HeLa cells increased. Both first and second mash with ginseng had $IC_{50}$ values against P388 and HeLa cells greater than 1 mg/mL, whereas the $IC_{50}$ values of fermented ginseng wine were 0.352 mg/mL for P388 cells and 0.713 mg/mL for HeLa cells. However, fermented ginseng wine did not exhibit cytotoxicity against A549 cells. The cytotoxic activities of rice wine without ginseng did not increase during fermentation. The ginsenosides found in ginseng may be prodrugs, which can be transformed to active compounds by acid or intestinal bacteria, and these transformed ginsenosides are more potent cytotoxic agents than ginsenosides in ginseng (24).

**Table 3. Active oxygen scavenging and antitumor activities of fermented ginseng wine**

| | $IC_{50}$ (mg/mL) | | | |
|---|---|---|---|---|
| | DPPH | P388 cells | HeLa cells | A549 cells |
| First mash with ginseng[1)] | 0.748 | >1 | >1 | >1 |
| Second mash with ginseng[1)] | 0.438 | >1 | >1 | >1 |
| Fermented ginseng wine[2)] | 0.394 | 0.352 | 0.713 | >1 |
| First mash without ginseng[1)] | >1.0 | >1 | >1 | >1 |
| Second mash without ginseng[1)] | 0.918 | >1 | >1 | >1 |
| Fermented rice wine[2)] | 0.884 | >1 | >1 | >1 |
| Caffeic acid | 0.003 | - | - | - |
| Cisplatin | - | 0.005 | 0.010 | 0.028 |

[1)]Day 0. [2)]Day 15.

In conclusion, traditionally fermented ginseng wine had a pleasant flavor which was developed by the various microflora in *nuruk*. Additionally, this fermented ginseng wine might have increased antioxidant and cytotoxic activities.

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