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Antioxidant Activities of Korean Rice Wine Concentrates

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ABSTRACT: The antioxidant activities of six Korean rice wine (KRW) concentrates were measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and lipid/malonaldehyde (MA) assays. In the DPPH assay, the antioxidant activities of the KRW concentrates, including *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ), were 40%, 66%, 64%, 35%, 35%, and 63%, respectively. Furthermore, the concentrates inhibited the formation of MA from cod liver oil by 49%, 83%, 75%, 82%, 89%, and 90%, respectively, according to the lipid/MA assay. The sample wines were also analyzed for pH, titratable acidity, soluble solids (°Bx), and reducing sugars. The antioxidant activities of volatile extracts of the KRWs extracted by a solvent assisted flavor evaporation (SAFE) apparatus were evaluated by aldehyde/carboxylic acid assay. The volatile extracts of MSJ, KHJ-1, KHJ-2, GGJ, SSJ, and SGJ inhibited the oxidation of hexanal by 97%, 99%, 90%, 90%, 50%, and 51%, respectively. Among the nonvolatile extracts of KRWs, KHJ-2 showed the highest inhibitory effect on MA formation.

KEYWORDS: Korean rice wines, concentrate, antioxidant, *Kookhwaju*, *Sogokju*

INTRODUCTION

The beneficial health effects of phytochemicals in alcoholic beverages have received much attention. In addition, the inhibitory effects of wine and beer on heart disease, arteriosclerosis, and cancer have been frequently reported.[1−6] The antimicrobial and anticancer activities of sake, Japanese rice wine, were also reported.[7] As interest in the functional properties of alcoholic beverages increases, many countries including Korea are making efforts to investigate the superiority of their traditional wines.

Various medicinal plants and herbs have been used as one of the raw materials for Korean rice wines (KRWs). Japanese apricot (*Prunus mume*), Lance Asiabell (*Codonopsis lanceolata*), Indian Dendranthema (*Dendranthema indicum*), and Chinese matrimony vine (*Lycium chinense Mill.*) have been widely used for *Maesilju* (MSJ), *Sasamju* (SSJ), *Kookhwaju* (KHJ), and *Gugijaju* (GGJ), respectively. For *Sogokju* (SGJ), glutinous rice is mainly utilized for alcohol fermentation. In addition, Indian dendranthema, malt, beans, and red pepper are also added to give SGJ its distinctive flavor.

These medicinal plants and herbs are used to enhance sensory qualities and provide various beneficial health effects to KRWs. The beneficial health effects of these medicinal plants were previously reported.[8−11] Japanese apricot, a raw material of *Maesilju* (MSJ), is known for its antioxidant and anticancer activities.[12−14] The effective antimicrobial and antioxidant activities of Dendranthema, which is widely used to make *Kookhwaju* (KHJ), have been reported.[15,16] Chinese matrimony is typically used as the base ingredient of *Gugijaju* (GGJ) and has been employed as an antifebrile agent.[17,18] The protective effect of Chinese matrimony against tetracarbon chloride-induced hepatotoxicity was previously reported.[19] *Sasamju* (SSJ) is fermented with the root of Lance Asiabell, and its antioxidant and anti-inflammatory activities were reported.[20,21]

Though the bioactivities of KRWs have been examined in various studies, no systematic approaches were reported to investigate the major compounds or fractions with beneficial effects. The majority of research on KRWs has focused on the biological activities of their nonvolatile extracts. In this study, the antioxidant activities of KRW concentrates were first measured, and then the volatile and nonvolatile extracts of the KRWs were separated by a solvent assisted flavor evaporation (SAFE) apparatus. The volatile and nonvolatile extracts of the KRWs were measured by two different assays including the aldehyde/carboxylic acid assay and lipid/malonaldehyde (MA) assay.

MATERIALS AND METHODS

Materials. Six Korean rice wines such as *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ) were obtained from a local store in Seoul. KHJ-1 and 2 were manufactured by different companies. Alcoholic contents of six samples were in similar ranges from 14 to 15% w/v.

Chemicals. Hydrogen peroxide, cod liver oil, trizma hydrochloride, and trizma base were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Dichloromethane, pentane, and ethyl acetate were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Hexanal (99%), undecane (99%) α-tocopherol (vitamin E; 95%), *N*-methylhydrazine (NMH), 2-methylpyrazine, sodium dodecyl sulfate (SDS), and ferrous chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Silicagel 60 N (particle size 63−200 μm: 70−230 mesh ASTM) anhydrous sodium sulfate was acquired from Kanto Chemical Co. (Tokyo, Japan). Amberlite XAD-2 resin was purchased from Supelco (Bellefonte, PA, USA)

Soluble Solid Content, pH, Titratable Acidity, and Reducing Sugar Contents of Korean Rice Wines (KRWs). The pH of samples was measured with a pH meter (Model 420A, Orion, USA) for 10 min at room temperature. Soluble solid content was determined by a hand refractometer (Model N-1E, Atago, Japan) at room temperature.

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Titratable acidity was assessed by following the AOAC titration method.[22] Approximately 5 mL of the KRWs was diluted with approximately 200 mL of distilled water followed by titration to pH 8.2 using 0.1 N NaOH. Titratable acidity was expressed as g succinic acid L^{-1}. The analyses were carried out in triplicate. Reducing sugars in the KRWs were estimated using dinitrosalicylic acid reagent, which consisted of 10 g of 3,5-dinitrosalicylic acid and 10 g of sodium hydroxide dissolved in 100 mL of distilled water, and then 0.5 g of sodium carbonate, 2 g of phenol, and 200 g of Rochelle salt were added, and the volume was made up to 1000 mL with distilled water. D-(+)-Glucose was used as a standard.[23] In the recommended procedure, 3 mL of reagent was mixed with 1 mL of the KRWs. The mixture was kept in a boiling water bath for 5 min at 65 °C and cooled immediately under the tap. The spectra of these solutions were taken by a UV/vis spectrophotometer (V-550, Jasco, Japan) at 550 nm. A linear glucose standard curve was used to calculate the results. The analyses were carried out in triplicate.

Preparation of Alcoholic Beverage Concentrates. Hundred milliliters of the alcoholic beverages including the KRWs was dealcoholated and concentrated by rotary evaporation under reduced pressure at 60 °C. The concentrate was then cooled at room temperature for 30 min. Finally, the concentrate was freeze-dried at −100 °C for 48 h using a freeze-dryer (Labconco Model 4451F, Kansas City, MO, USA), and stored at −20 °C until analyzed.

Isolation of Volatile and Nonvolatile Portions of KRWs Using SAFE. The volatile portions of the KRWs were isolated using a solvent assisted flavor evaporation (SAFE) apparatus.[24] One-hundred milliliters of the KRWs was poured into the distillation vessel of the SAFE apparatus. The volatiles were distilled and extracted at 45 °C under reduced pressure (10^{-3} Pa) using a rotary vacuum pump (Ulvac Technologies, Inc., Muthen, MA, USA). The volatile portion was collected in a distillate flask cooled by liquid nitrogen. After distillation, the distillate flask of the SAFE containing the volatile portion was thawed at room temperature and extracted by 50 mL of dichloromethane from aqueous solution (1 h). After extraction, the lower layer (dichloromethane) was separated from the upper layer (aqueous solution) using a separatory funnel. The lower layer was concentrated to 1 mL with a Vigruex column after drying over anhydrous sodium sulfate. The dichloromethane was removed using a purified nitrogen stream until the total volume was reduced to approximately 0.4 mL. The upper layer was poured into the unextracted materials, which remained in the distillation vessel containing the nonvolatile portion and was then concentrated to 1 mL using a rotary evaporator at 90 °C (R-124, Buchi, Switzerland).

DPPH Radical Scavenging Assay. The free radical scavenging activities of the KRWs were measured by the DPPH assay. The DPPH method was employed because this technique is easily applicable and widely used. DPPH is a synthetic free radical that accepts an electron or hydrogen to be converted to a stable DPPH molecule. The disappearance of DPPH radicals was monitored by a decrease in the absorbance of a solution at 517 nm. The DPPH solution (1.52×10^{-7} M) was prepared in a mixture of water and ethanol (1:1). A 4.5 mL amount of DPPH solution and 0.5 mL of KRW extracts (1000 μg/mL) were mixed using vortex and stored in a dark place for 20 min. After the reaction, absorption activity was measured using a UV−vis spectrophotometer (Shimazu, Kyoto, Japan) at 517 nm. Fifty percent ethanol (w/v) was treated as a control and free radical scavenging activity was calculated as shown below.

$$\text{Inhibition percentage}\ (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

For comparison purposes, α-tocopherol (1000 μg/mL) was examined using the same methodology used for antioxidant activity.

Aldehyde/Carboxylic Acid Assay. To measure the antioxidant activities of the volatiles in the KRWs, fractions of the volatile portions and authentic phenolic chemicals in the volatile fractions were examined using the aldehyde/carboxylic acid assay.[25,26] The aldehyde/carboxylic acid test is a fast and simple method to assess the antioxidative properties of chemicals or groups of chemicals. This method is based on the autoxidation of aldehydes to carboxylic acids with active oxygen species such as hydroxyl radicals.[27] The volatile extracts (100 μg/mL) were added to a 2 mL dichloromethane solution of hexanal (3 mg/mL) containing 0.2 mg/mL undecane as a GC internal standard. The oxidation of the sample solution was initiated by heating at 60 °C for 10 min in a sealed vial followed by storage at room temperature. The headspace of each vial was purged with pure air (1.5 L/min, 3 s) every 24 h for the first 10 days. The decrease in hexanal was monitored at 10-day intervals. α-Tocopherol (100 μg/mL) was also examined for its antioxidant activity using the same methodology.

Lipid/MA (Malonaldehyde) Assay. The antioxidant activities of nonvolatile portions of the KRWs were determined by analyzing MA formed from cod liver oil upon oxidation after derivatizing to 1-MP (methylparazine) with NMH (*N*-methylhydrazine).[28] An aqueous solution (5 mL) containing 500 μL of tested sample, 30 μL of cod liver oil, 0.25 mmol of trizma buffer (pH 7.4), 5 mmol of ferrous chloride, 10 mmol of hydrogen peroxide, 0.75 mmol of potassium chloride, and 0.2% of surfactant SDS was incubated with various volatile extracts (300 μg/mL) for 17 h at 37 °C in a 20 mL test tube. The oxidation of samples was stopped by adding 50 μL of a 4% BHT solution. A known antioxidant, α-tocopherol, was used to compare its antioxidant activity to that of the aroma extracts tested. NMH (30 μL) was added to the oxidized cod liver oil solutions, and the solutions were stirred for 1 h at room temperature. The extract was adjusted to exactly 10 mL by adding ethylacetate and 20 μL of a 2-methylpyrazine solution as a GC internal standard. The solution was analyzed for 1-MP by a gas chromatograph with a nitrogen−phosphorus detector (NPD).

Instrumental Analysis. The quantitative analysis of hexanal was conducted according to a previously reported internal standard method.[25] A Hewlett-Packard (HP) model 6890 GC equipped with a 30 m × 0.32 mm i.d. (d_f = 0.25 μm) HP-1 bonded-phase fused-silica capillary column (Agilent Technologies, Wilmingtion, DE, USA) and an FID was used for hexanal analysis. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 20:1. The injector and detector temperatures were 300 and 280 °C, respectively. The oven temperature was programmed from 40 to 180 °C at 4 °C/min and held for 10 min. A HP model 6890 GC equipped with a 30 m × 0.32 mm i.d. (d_f = 0.25 μm) DB-WAX bonded phase fused-silica capillary column (J & W Scientific, Folsom, CA, USA) and an NPD were used for analysis of 1-MP.

RESULTS AND DISCUSSION

Soluble Solids, pH, Titratable Acidity, and Reducing Sugar Contents of Korean Rice Wines (KRWs). The general chemical compositions of the rice wine samples are shown in Table 1. The pH level of the six samples ranged from 3.51 to 4.59. In another study of 22 Korean traditional fermented rice wines, pH levels similarly ranged widely from 3.43 to 4.5.[29] Because malt was added before fermentation, the Brix and reducing sugar levels in SGJ were elevated and were the highest among the six samples. Reducing sugar levels in the other KRWs were similar, ranging from 0.23 to 1.94. The titratable acidity of MSJ was the highest compared to that of the other rice wines. This high level of acidity could be attributed to the addition of Japanese apricot. Except for MSJ, titratable acidity ranged from 3.57 to 6.68 in the other samples.

Antioxidant Activities of KRW Concentrates. The antioxidant activities of the KRWs were measured by DPPH radical scavenging activity assay and lipid/MA assay. For comparison

purposes, α-tocopherol was examined for antioxidant activity by the same methodology. After freeze-drying, 100 mL of KRWs were concentrated to 0.22−4.33 g. The yield of freeze-drying was between 2.27 mg/mL and 43.30 mg/mL for the KRWs. The DPPH radical scavenging activities of all of the KRWs are shown in Figure 1. The inhibition activity of KHJ-1 was 66%, presenting the highest activity. KHJ-2 and SGJ showed 63% and 64% inhibition activity, respectively. MSJ and GGJ both showed 40% inhibition activity. SSJ showed the lowest inhibition activity of 35%. KHJ-1, KHJ-2, and SGJ showed inhibition activities greater than the inhibition activity of α-tocopherol (1000 μg/mL).

The antioxidant activities of all KRW concentrates as measured by the lipid/MA assay are shown in Figure 2. The lipid/MA assay is specific to measure a lipid peroxidation product, namely, MA, in samples. α-Tocopherol (300 μg/mL) inhibited the formation of MA by 93%. SGJ and SSJ showed the highest inhibition activities against MA formation at 90% and 89%, respectively. KHJ-2, KHJ-1, and GGJ showed 75%, 83%, and 82% inhibition activity, respectively. MSJ showed the lowest inhibition activity toward MA formation at 49%. KHJ-1, KHJ-2, and SGJ showed higher antioxidant activities in both the DPPH

Table 1. Soluble Solids (°Brix), pH, Titratable Acidity, and Reducing Sugar Contents of KRWs[a]

samples	pH	BRIX[a] (°)	TA[b] (g L^{-1})	RS[c] (g 100 mL^{-1})
SGJ	4.59 ± 0.02	19.70 ± 0.00	6.47 ± 0.07	4.36 ± 0.04
SSJ	4.48 ± 0.04	10.80 ± 0.00	3.69 ± 0.17	1.94 ± 0.01
GGJ	3.61 ± 0.01	6.13 ± 0.06	5.3 ± 0.22	0.23 ± 0.00
KHJ-1	3.55 ± 0.01	8.63 ± 0.15	6.68 ± 0.86	1.04 ± 0.00
KHJ-2	3.51 ± 0.01	9.77 ± 0.12	3.57 ± 0.16	1.33 ± 0.01
MSJ	3.55 ± 0.02	7.17 ± 0.12	8.02 ± 0.10	0.39 ± 0.00

[a] Values represent the means of triplicate determinations ± SD. a, soluble solid contents; b, titratable acidity expressed as g succinic acid/L; c, reducing sugar contents.

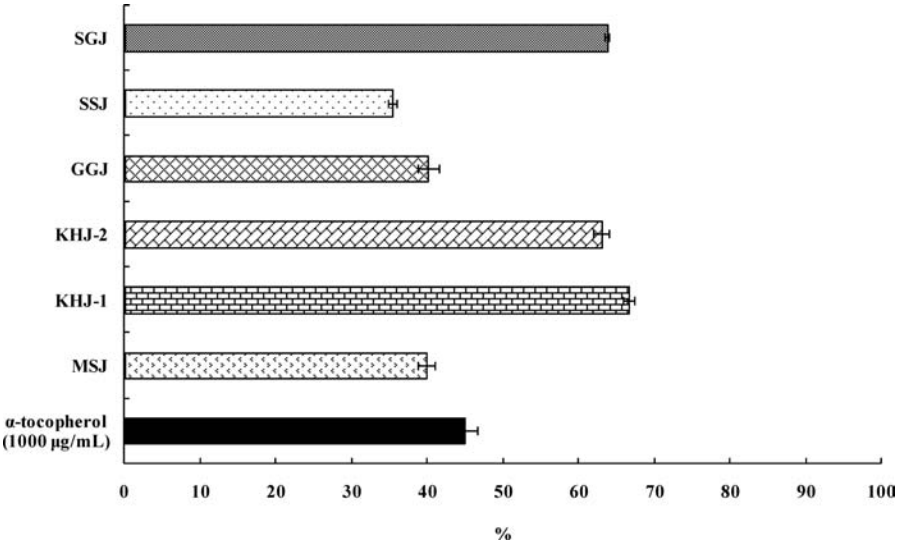

Figure 1. Radical scavenging capacity (%) of various Korean rice wine (KRW) concentrates and α-tocopherol (1000 μg/mL): *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ).

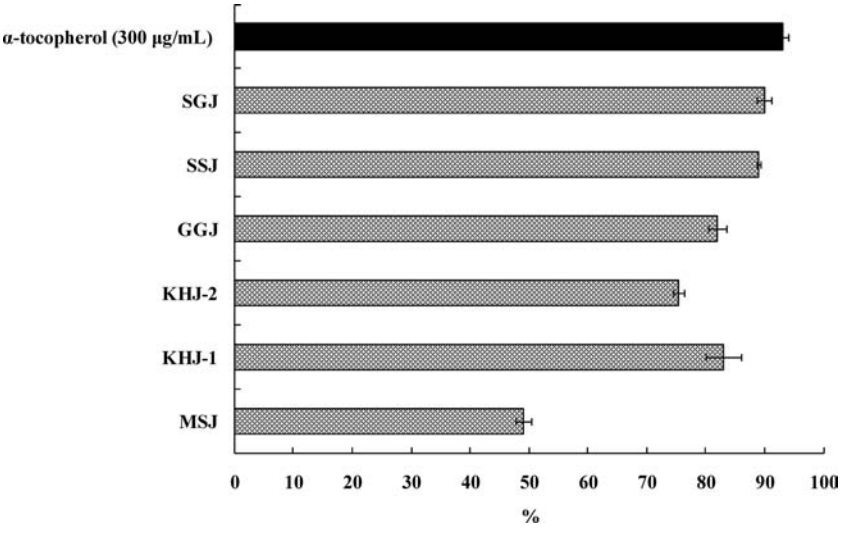

Figure 2. Inhibitory effects (%) of KRW concentrates and α-tocopherol (300 μg/mL) toward malonaldehyde formation from cod liver oil; *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ).

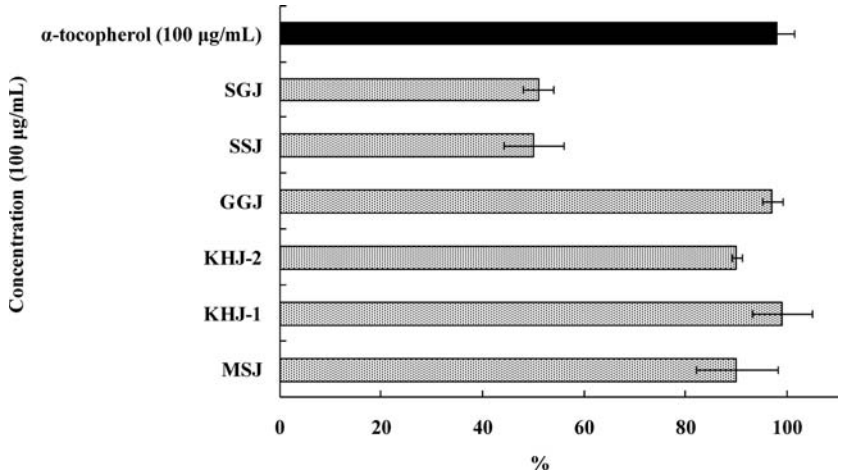

Figure 3. Antioxidant activity (%) of volatile portions of KRWs and α-tocopherol (100 μg/mL) measured by the aldehyde/carboxylic acid assay; *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ).

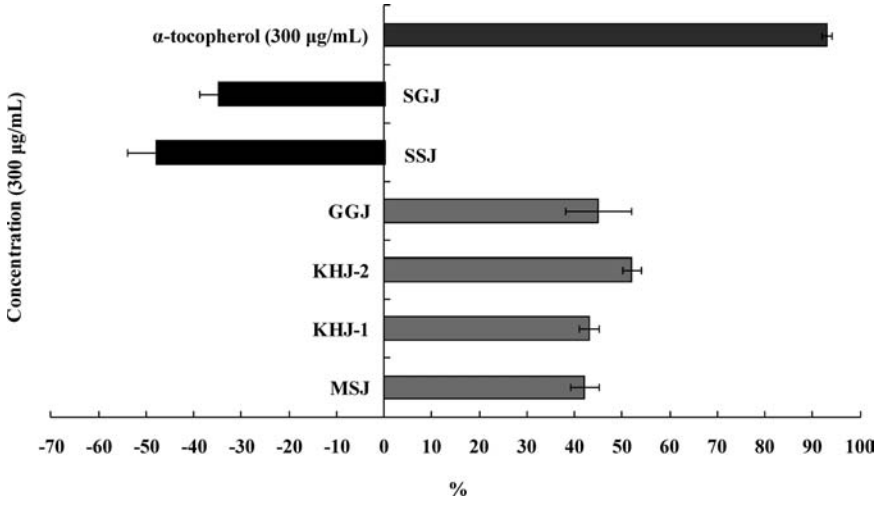

Figure 4. Inhibitory effects (%) of nonvolatile portions of KRWs and α-tocopherol (300 μg/mL) toward MA formation from cod liver oil; *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ).

and lipid/MA assays. The antioxidant activities of KHJ and MSJ were tested in various systems in previous reports. The antioxidant activity of KHJ was higher than that of MSJ with respect to radical scavenging activity.[30] *Compositae* plants like the raw materials of KHJ have been previously reported to possess strong antioxidant activities, and the extract of *Artemisia capillaries* was reported to have the highest effects in two oxidation inhibition assays.[31] The antioxidant activity of *Prunus mume*, a raw material of *Maesilju* (MSJ), has been reported several times and tested by various methods.[32,33] The antioxidant activities of *Lycium chinese* and *Dendranthema indicum*, respective raw materials of *Gugijaju* (GGJ) and *Kookhwaju* (KHJ), were also reported.[34,35] However, only a few studies exist on the antioxidant activity of KRWs, in contrast to numerous studies on that of plant materials used in KRWs.

Antioxidant Activities of Volatile Portions of KRWs by the Aldehyde/Carboxylic Acid Assay. The antioxidant activities of volatile extracts of all KRWs, as measured by the aldehyde/carboxylic acid assay, are shown in Figure 3. KHJ-1 showed the highest antioxidant activity at 99%. The antioxidant activities of MSJ, GGJ, and KHJ-2 were 90%, 97%, and 90%, respectively. The antioxidant activities of SSJ and SGJ were 50% and 51%, respectively. There have been many studies regarding the characterization of volatile components related to the quality control of several red and yellow rice wines.[36,37] So far, the antioxidant potentials of volatile extracts from rice wines have rarely been studied. The rice wines in reported studies are limited to Chinese or Japanese rice wines. The main research objective of the studies was to determine the profile of volatile compounds in the rice wines. Que et al. reported the antioxidant properties of the concentrate and volatiles of Chinese yellow rice wine.[38] Chen and Xu reported the influence of yeast strains on the volatile flavor compounds of Chinese rice wine.[39]

Inhibitory Effects of Nonvolatile Compounds in KRWs toward MA Formation from Cod Liver Oil. The antioxidant activities of nonvolatile compounds in all the wines, as measured by lipid/MA assay, are shown in Figure 4. Lipid oxidation proceeded to 48% and 35% in SSJ and SGJ, respectively. However, KHJ-2 (50%) had the highest antioxidant activity. MSJ, GGJ, and KHJ-1 showed inhibitory effects of 42%, 43%, and 42%, respectively. The antioxidant activity of α-tocopherol (300 μg/mL) was 93%. Many studies have dealt with the antioxidant

activities of Korean rice wines.[29,30,40] In these reports, the antioxidant activities of the rice wines were mainly contributed by polyphenolic compounds and/or flavonols. In addition, polysaccharides and polysaccharide−peptide complexes in the rice wines attributed various beneficial bioactivities.[41] The determination of nonvolatile compounds in rice wines from the present study is currently underway in our laboratory.

In conclusion, KRWs such as *Maesilju* (MSJ), *Kookhwaju*-1 (KHJ-1), *Kookhwaju*-2 (KHJ-2), *Gugijaju* (GGJ), *Sasamju* (SSJ), and *Sogokju* (SGJ) showed antioxidant activity in the DPPH assay. In terms of general chemical composition, SGJ showed a different profile compared to those of other KRWs since it was higher in soluble solids and reducing sugar content. By the addition of Japanese apricot, MSJ showed the highest level of titratable acidity. Radical scavenging activity and inhibitory activities toward MA formation were found in the KRWs. Also, in the volatile and nonvolatile portions of the KRWs, antioxidant activities were shown. In the future, antioxidant activity measurements of the volatile and nonvolatile fractions will be carried out. These results will illustrate in more detail which chemical species and fractions contribute antioxidant activity to the KRWs.

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