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Antioxidant Activities of Aroma Extracts in Commercially Available Red Wines in Korea

– Research Note –

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Abstract

The antioxidant activities of aroma extracts from commercially available red wines in Korea were evaluated. The aroma extracts of the red wines were extracted by simultaneous steam distillation. Antioxidant activity was measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical cation scavenging activity. DPPH radical scavenging activity of the aroma extracts in the red wines increased with increases in the amount of wine used for aroma extraction. Antioxidant activities of domestic wine 1, imported wine 7, and imported wine 12 were 97.16, 96.72 and 94.52%/20 mL wine by DPPH assay and 7.09, 8.07 and 7.28 mg ascorbic acid equivalents per mL wine by ABTS assay, respectively. This study demonstrates potent antioxidant activities of the aroma extracts of commercially available red wines in Korea.

Key words: red wine, aroma, simultaneous steam distillation extraction, antioxidant activity

INTRODUCTION

The amounts and types of phenolics present in grape juice and wines may play an important role in controlling oxidative stress in the human body. Wines contain a wide range of polyphenolic constituents that have been reported to possess anticancer and anti-inflammatory effects as well as the ability to block cellular events predisposing to atherosclerosis and coronary heart disease (CHD) (1-4). In France, CHD mortality is lower than in other industrialized countries, even though the dietary intake of saturated fat is higher. Regular consumption of red wine has been hypothesized to be the most likely cause for this phenomenon known as the "French Paradox" (1,5-7).

Grape volatile compounds are the main contributors to the fresh and fruity note of wines. Compounds responsible for this aroma (terpenes, C13-norisoprenoids, benzene derivatives, and aliphatic alcohols) are present in grapes, mainly in the skin. Concentrations of these volatile compounds are different depending on the grape variety, cultural practice, and climatic or biological factors (8). Volatile compounds are one of the most important sensory attributes of fruits and vegetables, and flavors are particularly sensitive to compositional alterations (9). The volatile compounds that contribute to the flavors of fruits and vegetables are produced through metabolic pathways during ripening, harvest, postharvest, and storage; and are influenced by many factors related to species, variety, and technological treatments (10). Recent studies have revealed potent antioxidant activity of natural volatile compounds, especially those from fruits and vegetables (11,12).

The aroma compounds contribute important sensory characteristics in wines and they influence the quality of wines. However, no studies have determined the antioxidant activity of aroma compounds in the wine. The objective of this study was to determine antioxidant activities of the aroma extracts of various wines by simultaneous distillation and extraction (SDE).

MATERIALS AND METHODS

Chemicals and wine samples

Aascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), diammonium salt of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), α-pinene, and potassium persulphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Diethyl ether was purchased from Wako Pure Chemical (Osaka, Japan). All other reagents and solvents used were of analytical and HPLC grade. The wines samples were purchased from a local market

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in Korea. Four different domestic and twelve different imported red wines were tested in this study.

Extraction of aroma compounds

The extraction of aroma compounds was done by the method of Woo et al. (11) and Jeong et al. (12) with some modifications. The wine (300 mL) with the addition of distilled water (700 mL) was extracted with 50 mL of diethyl ether for 2 hr using a Likens-Nickerson type simultaneous distillation and extraction (SDE; 13) apparatus (Kontes, Vineland, NJ, USA). The extract was dried over anhydrous sodium sulfate and the solvent was then concentrated to 1 mL with a gentle stream of nitrogen gas (99.99% purity). Antioxidant activities were measured in the extracts diluted in diethyl ether.

DPPH radical scavenging activity

The DPPH radical scavenging activity was estimated using the method described by Tepe et al. (14) and Lee et al. (15) with some modifications. Aliquots (0.8 mL) of 0.2 mM DPPH (Sigma, St. Louis, MO, USA) in methanol were mixed with 0.2 mL of the diluted extracts. The mixtures were vigorously shaken and left to stand for 30 min under subdued light. The absorbance at 520 nm was measured against diethyl ether as a blank and converted into percentage antioxidant activity using the following formula:

Electron donating activity (EDA)=[1 − (absorbance of sample at 520 nm)/ (absorbance of control at 520 nm)] $\times$100.

The mean values were obtained from triplicate measurements.

ABTS radical scavenging activity

The ABTS radical scavenging activity was estimated using the method of Re et al. (16) and Woo et al. (17) with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulphate solution and the mixture was left overnight in the dark at room temperature. The ABTS radical cation solution was diluted with methanol to obtain an absorbance of 1.4 to 1.5 at 735 nm (molar extinction coefficient, e=3.6×10^4 $mol^{-1}cm^{-1}$). Diluted ABTS radical cation solution (1 mL) was added to 50 μL of the extract or ascorbic acid standard solution. The absorbance at 735 nm was determined using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan) after 30 min. The ABTS cation scavenging activity was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) and defined as the milligrams of ascorbic acid equivalents per milliliter sample. The AEAC was calculated by the following equation:

$$AEAC = (\Delta A_{sample} \ / \ \Delta A_{aa}) \times C_{aa} \times V \times (1 \ / \ W_{sample}),$$

where ΔA_{sample} is the change of absorbance in the presence of sample, ΔA_{aa} is the change of absorbance after addition of ascorbic acid standard solution, C_{aa} is the concentration of ascorbic acid standard solution (mg/mL), V is the volume of sample (mL) and W_{sample} is the weight of sample (mL). All extracts were analyzed in triplicate.

RESULTS AND DISCUSSION

DPPH radical scavenging activity of aroma extracts

Radical scavengers in the wine aroma extracts were evaluated by reactivity toward a stable free radical, DPPH. The antioxidant activity (EDA, %) of the aroma extracts of various wines by SDE method, as determined by scavenging DPPH radical, are presented in Fig. 1. The DPPH radical scavenging activities of the aroma extracts varied from 11.33 to 57.18% from 10 mL of the wines, 12.03 to 97.16% with aroma extracts from 20 mL of the wines, and from 17.00 to 97.78% with aroma extracts from 30 mL wines. DPPH radical scavenging activity of domestic wine 1, imported wine 7, and imported wine 12 were highest at 97.16, 96.72 and 94.52%/20 mL wine, respectively.

ABTS radical scavenging activity of aroma extracts

The ABTS radical scavenging activities of the aroma extracts of various wines, as determined by scavenging ABTS radical, are presented in Fig. 2. The ABTS radical scavenging activity of the aroma extracts of various wines are expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as the mg of ascorbic acid equivalents per mL wine. The ABTS radical scavenging activity of the aroma extracts of various wines showed between 0.0239 and 8.0720 mg ascorbic acid equivalents per milliliter wine. ABTS radical scavenging activity of domestic wine 1, imported wine 7, and imported wine 12 were highest at 7.09, 8.07, and 7.28 mg ascorbic acid equivalents per milliliter sample, respectively.

The differences in antioxidant activities between wine aroma extracts could be explained by differences in the amounts of antioxidant compounds. The occurrence of antioxidant compounds in red wines is not only a consequence of their extraction from grapes during the wine-making process or the use of different grape varieties. Other important influencing factors are the viticultural area, the vintage, the time at which grapes are picked, and the storage time of the wine bottles. Several condensation reactions which affect especially anthocyanins, catechins and procyanidins take place over the shelf-life of red wines, resulting in decreased levels of

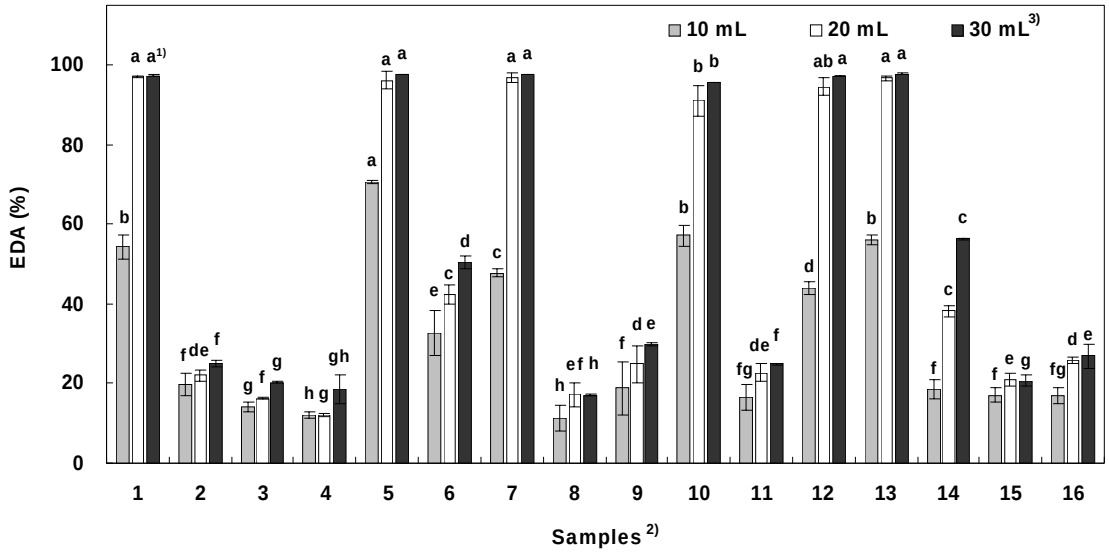

Fig. 1. DPPH radical scavenging activity (EDA) of the aroma extracts of various wines (n=3).
1)Any means in the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test. 2)Different domestic red wines: 1 to 4, different imported red wines: 5 to 16. 3)Amount of wine tested.

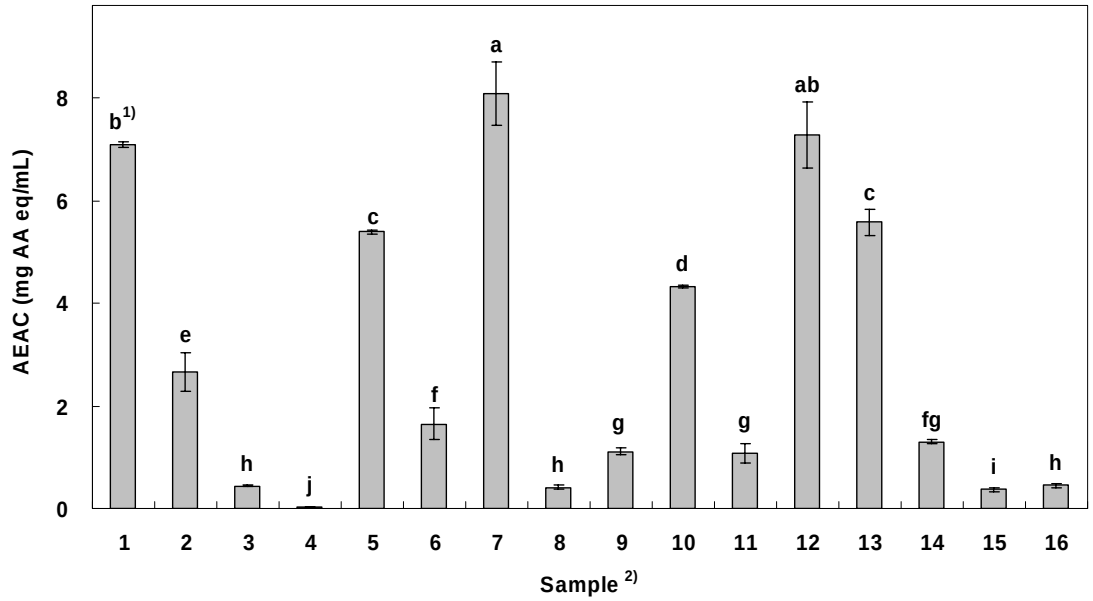

Fig. 2. ABTS radical cation scavenging activity of the aroma extracts of various wines (n=3).
1)Any means in the same column followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test. 2)Different domestic red wines: 1 to 4, different imported red wines: 5 to 16.

these substances and in the formation of new polymeric pigments (18,19). Because of this, a large number of influencing factors affect the final concentration of antioxidant compounds making their content quite variable in red wines. Woo et al. (11) reported that the ABTS radical scavenging activity of raw garlic aroma extract, expressed as AEAC and defined as the mg of ascorbic acid equivalents per g of garlic, was 39.05 mg ascorbic acid (AA) equivalents (eq)/g sample. After heating, the AEAC values were 45.09～46.43 mg AA eq/g sample. The antioxidant activity of volatile compounds extracted from heated garlic increased with increasing heating temperature and time. Jeong et al. (12) reported that antioxidant activity of garlic volatile compounds produced an effect proportional to the amount of sulfur compounds such as diallyl disulfide, methyl-2-propenyl trisulfide and di-2-propenyl trisulfide. Therefore, in this study, antioxidant activities of the volatile compounds in domestic and imported red wines were evaluated, and revealed potent antioxidant activities of the aroma extracts of

commercially available red wines in Korea.

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