

Enhancing the Antioxidant Activities of Wines by Addition of White Rose Extract

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White rose petal extract (WRE) contains large amounts of phenolic compounds and is considered edible. In this study, red and white wines were prepared by the addition of WRE (0.10% or 0.25% (w/v)), followed by fermentation at 25°C for 15 days. The fermentation profiles, colors, sensory test results, and antioxidant activities of the wines were compared. As reported herein, the fermentation profiles of the pH, CO₂ production rate, and final ethanol concentration were not affected by the addition of WRE, but a slow consumption rate of sugar was observed in 0.25% WRE-added wine. In contrast, the total polyphenol concentrations in WRE-added wines increased significantly ($p < 0.05$) in a dose-dependent manner, resulting in appreciable enhancement of the antioxidant activities of the wines. Chromaticity tests showed slight changes in the redness and yellowness, but sensory tests showed that the overall flavor qualities of the WRE-added wines were acceptable to the panels. This study demonstrates that addition of WRE to wine confers beneficial health effects and this treatment results in better outcome in white wine.

Keywords: White rose petal extract, wine, antioxidant activity, DPPH, ABTS

Introduction

Rose trees are cultivated to produce flowers and the raw material of essential oils. Rose petals have been used in herbal and folk remedies for cancer, blood circulation disease, and menstrual problems [1]. Rose petals contain phenolic compounds such as gallic acid, rutin, kaempferol, quercetin, and myricetin [2]. Rose extract effectively soothes skin exposed to heat [2]. In particular, white rose petal extract (WRE) has shown several beneficial health effects, such as antioxidation, anti-*Helicobacter*, antiallergenic, and skin-whitening activities [3, 4]. Currently, phenolic compounds extracted from the rose petals are used to make functional beverages in the food industry [5].

Moderate long-term intake of red wine is known to lower the risk of cardiovascular disease [6], arteriosclerosis [7], and cancer [8]. In addition, consumption of wine may be helpful in reducing the occurrence of neurological disorders

such as dementia and Parkinson's disease, caused by oxidative stress [9]. However, the beneficial health effects of wine may differ depending on wine type (red or white) or grape variety, because the phytochemical concentrations of the grapes used are very diverse. Wines contain polyphenols such as phenolic acids, stilbenes, and flavonoids [10]. Phenolic compounds in red wine are derived from the grape skin, as well as from grape seeds, stems, and pulp, all of which are important sources of the flavanols transferred to the wine during fermentation [11]. In contrast, white wines are usually made from free-running juice; without the grape mash, there is no contact with the grape skins [12], which is thought to be the main reason for the relatively low polyphenol contents and lower antioxidant activity [13]. The total polyphenol contents also vary with the grape variety; Carbenet Fran and Pinot Noir—mainly used for red wine fermentation—have polyphenol contents of 396–424 mg/ml, whereas Campbell Early, often used in

Korea, has a polyphenol content of 110 mg/ml [14, 15].

In this study, in order to increase the antioxidant activities of wine, WRE was added to grape must and the effects of WRE addition on wine fermentation were investigated. For this, WRE was extracted from white rose petals with ethanol and dried to a powder, and 0.1% or 0.25% WRE was added to red grape must (Campbell Early grape) or green grape juice followed by fermentation at 25°C for 15 days. For these four different wines, fermentation profiles (gas production, °Brix, and pH), changes in color, antioxidant activities, and sensory acceptance were analyzed.

Materials and Methods

Materials

For red wine, Campbell Early grape (20.2 °Brix) was used (Yeongdong-gun, Chungbuk Province, Korea, 2015) and for white wine, green grape extract (Nongshim Inc., Korea) was used. For the starter culture, *Saccharomyces cerevisiae* Lalvin EC-1118 (Lallemand Inc., Canada) was inoculated.

Extraction of White Rose Petals

Fresh white petals of *Rosa hybrida* Colorado, cultivated in a grange (Rose Rangs, Korea, 2015), were harvested. After drying, they were ground in a rotor speed mill (Laval Lab Inc., Canada), and the crushed petals were disinfected with 70% ethanol spray, followed by drying at 80°C for 24 h. The dried white rose petal powder was extracted with 50% ethanol at 60°C for 3 h, and the extract was completely dried to obtain WRE by using a vacuum evaporator (Büchi 461; Büchi, Switzerland).

Winemaking by Addition of WRE

For red wine fermentation, destemmed and crushed grape berries were transferred into three fermenter jars (2 L). Sucrose (CJ, Korea) and sodium metabisulfite (Sigma, USA) were added to attain 24 °Brix and 100 ppm samples, respectively. For white wine fermentation, green grape juice was divided into two fermenter jars and sucrose was added to make a 24 °Brix sample. In the sample jars of red or white wine, WRE was added to make 0.10% (w/v) or 0.25% (w/v) solutions. Fermentation was initiated by adding a rehydrated inoculum of *S. cerevisiae* (10⁷ CFU/ml) and the resulting mixture was incubated at 25°C for 14 days. For comparison of the chromaticity and sensory analysis results, WRE was also added to unmodified wine samples after completion of fermentation.

Chemical Analyses

The pH was measured by a calibrated pH meter (pH-200; Istek, Korea). The concentration of total soluble solids (°Brix) was measured by a refractometer (Atago, Japan). The concentrations of gallic acid in the wines were analyzed using an HPLC system (Agilent 1260 Infinity; Agilent Technology, USA) equipped with a

ZORBAX SB-C18 column (4.6 × 150 mm; Agilent Technology). Water/85% *o*-phosphoric acid (99.5/0.5 (v/v)) (solvent A) and acetonitrile/water/85% *o*-phosphoric acid (50/49.5/0.5 (v/v/v)) (solvent B) were used as the mobile phases. Gradient elution was performed with 90% solvent A and 10% Solvent B for 5 min, 18% solvent A and 82% solvent B from 5.1 to 33 min, and 90% solvent A and 10% solvent B from 33.1 to 38 min. The flow rate of the mobile phase was 1.0 ml/min and the absorbance of gallic acid was monitored at 255 nm using a UV detector at a column temperature of 25°C. The alcohol concentration in the wines was measured by densitometry at 15°C after recovering the alcohol fraction using a distiller [16].

Total Polyphenol Content

Total phenolic contents of the wine were determined by the Folin–Ciocalteu colorimetric method [17]. Total phenolics were expressed as gallic acid equivalents (mg GAE/ml).

Antioxidant Activities

The antioxidant activity of the wine was measured by comparing the antioxidation level of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) measured by the method proposed by Maruthamuthu *et al.* [18]. In brief, a 1.0 ml solution of the wine at different concentrations (100%, 10%, and 1% (v/v)) in distilled water was mixed with 1.0 ml of ABTS (0.2 mM). After 30 min incubation at room temperature, the absorbance was measured at 563 nm using a spectrophotometer (PowerWave; BioTek Co., Korea). Decolorization levels were calculated according to the following equation:

$$\text{Antioxidant activity (\%)} = [1 - A/B] \times 100 \quad (1)$$

where A is the absorbance of the wine sample and B is the blank absorbance.

In addition, the antioxidant activity of the wine was also measured by comparing against the antioxidation level of 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the method proposed by Shimada *et al.* [19]. In brief, a 1.0 ml solution of the wine at different concentrations (100%, 10%, and 1% (v/v)) in methanol was mixed with 1.0 ml of a methanolic solution of DPPH (0.2 mM). After strong shaking followed by standing at room temperature for 30 min, the absorbance was measured at 517 nm against a methanol blank. Decolorization levels were calculated according to the above-mentioned Eq. (1).

Chromaticity and Sensory Analyses

Tristimulus values of color parameters—*L* (lightness), *a* (redness), and *b* (yellowness)—were determined for the wines using a colorimeter (CM-3500d; Konica Minolta, Japan). Sensory evaluation of the wines was carried out by a panel comprising 40 individuals from the Department of Food Science and Biotechnology at Chungbuk National University and was based on the aroma, color, sweetness, tartness, astringency, and overall acceptability, using a 9-point verbal hedonic scale (1 = extremely disliked, 5 =

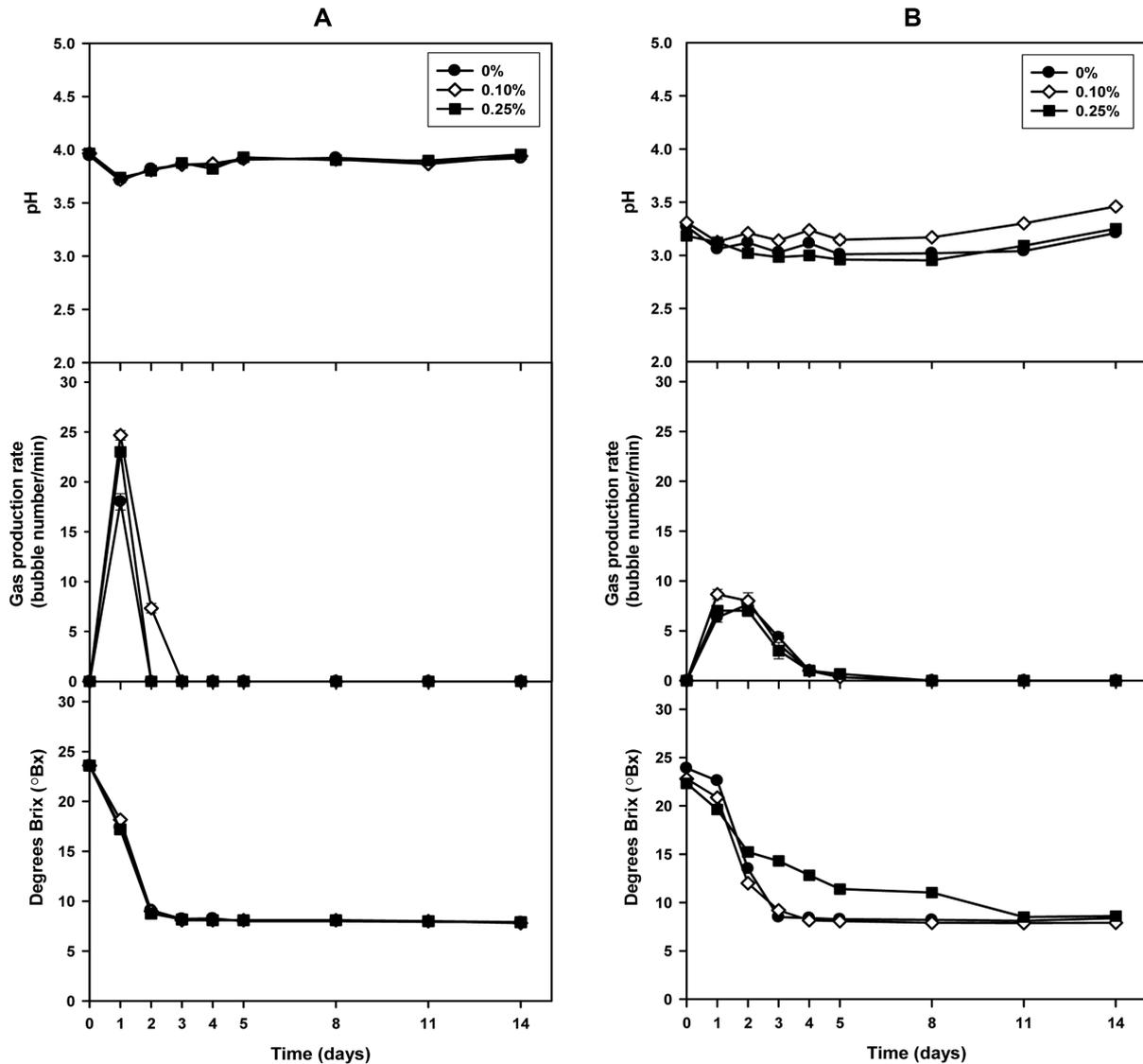


Fig. 1. Changes of pH, °Brix, and gas production rate during fermentation of red wine (A) and white wine (B) containing white rose petal extract (0%, 0.10%, or 0.25%).

$n = 3$, Error bars show standard deviation.

neither liked nor disliked, 9 = extremely liked). Each wine sample was assigned a 3-digit random code and 20 ml wine samples were provided in transparent wine glasses.

Results and Discussion

Monitoring of Alcohol Content, pH, and Brix Changes during Wine Fermentation

Chemical characteristics of wine—namely, the pH, °Brix, and gas production rate—were monitored after addition of 0.1% or 0.25% WRE (Fig. 1). During the fermentation

period of red and white wines, the pH was maintained at 3.71–3.96 for red wine and 2.96–3.46 for white wine. In white wine, addition of WRE resulted in a slight decrease in the pH, but the overall fermentation pattern was not different between the wines. Gas production rates increased immediately after inoculation of the starter and continued for 2–3 days in red wine and for 5 days in white wine, showing a slower fermentation rate in white wine. The initial brix levels (24 °Brix) dropped to reach a plateau (8 °Brix) in 3 days in red wine and in >5 days in white wine, showing a slower consumption rate of sugars in white

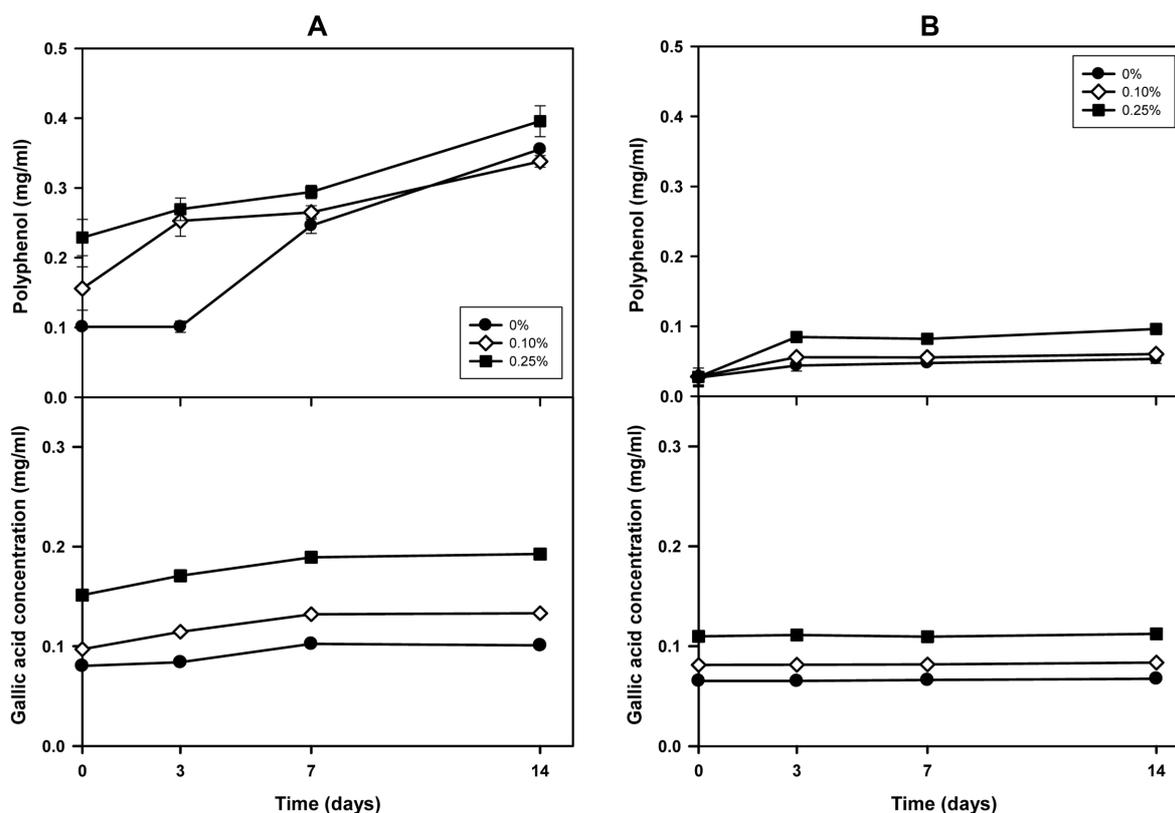


Fig. 2. Changes in total polyphenol and gallic acid concentrations during fermentation of red wine (A) and white wine (B) containing white rose petal extract (0%, 0.10%, or 0.25%).

$n = 3$, Error bars show standard deviation.

wine. The fermentation profile observed in Fig. 1 is consistent with the previous report of fermentation with Campbell early grape [16]. In particular, in white wine showing lower initial pH, addition of 0.25% WRE resulted in slower depletion of soluble sugars (consumed in 11 days) compared with the other samples, revealing an inhibitory effect of acidity and WRE (0.25%) on ethanol fermentation of yeast. However, alcohol concentrations were measured after 14 days as 12.2% for red wines and 11.5% for white wines. This result showed that addition of WRE did not have a deteriorating effect on the fermentation profiles of either red or white wines.

Total Polyphenol and Gallic Acid Contents

Concentrations of total polyphenols and gallic acid were analyzed in the wines. As shown in Fig. 2, the initial concentrations of total phenolic compounds in red wines were 0.1–0.25 mg/ml and their levels increased with fermentation. This has been often observed during red wine fermentation [20, 21] and could be attributed to the

hydrolysis of tannic substances by tannase [22, 23] as well as diffusion of soluble phenolic compounds from grape peel. In contrast, in white wine, the phenolic compound levels were almost constant (between 0.08 and 0.10 mg/ml) throughout fermentation, which could be attributed to the absence of grape peel in the wine liquid. As the WRE content in the wines was increased, the total polyphenol concentrations also increased with respect to the amount added in both red and white wines. Fig. 2 also shows the concentration changes of gallic acid in wines and the results are similar to those of the total polyphenol contents; the gallic acid content increased with red wine fermentation, but it was constant in white wine. The addition of WRE resulted in an increase in the gallic acid content of both wines. Sato *et al.* [24] reported that the phenolic content of red wines from different sources ranged between 0.736 and 2.858 mg/ml and that of white wines was in the range 0.259–0.721 mg/ml. Considering the phenolic contents of WRE, which is 243.5 mg gallic acid equivalent/g dry mass [3], addition of 0.25% WRE (0.61 mg gallic acid equivalent/ml)

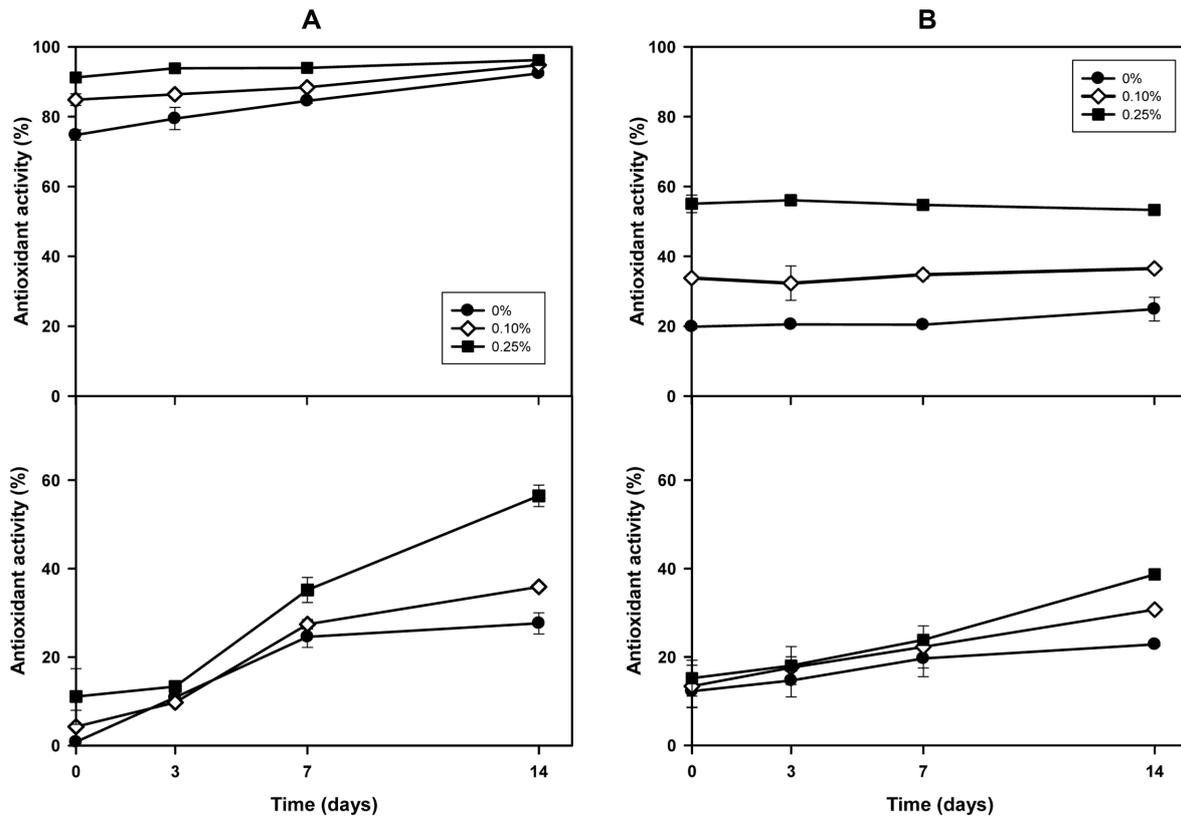


Fig. 3. Changes in ABTS and DPPH activities during fermentation of red wine (A) and white wine (B) containing white rose petal extract (0%, 0.10%, or 0.25%).

$n = 3$, Error bars show standard deviation.

in red and white wines resulted in 83% and 235% of maximal increase, respectively, in the concentration of total phenol compounds. Hence, our result shows that the polyphenolic contents of wines can be fortified by simple addition of WRE and the effect would be more dominant in white wine.

Antioxidant Activity

In order to compare the antioxidant activities of red and white wines according to the concentration of WRE, ABTS and DPPH assays of the wine samples were performed (Fig. 3). The ABTS assay showed that the antioxidant activity was high in red wines and their capacities increased with increased addition of WRE in both wines. The DPPH assay demonstrated that the antioxidant capacities of red and white wines increased gradually with the fermentation period, and their levels varied with the amount of WRE added. Results illustrated in Figs. 2 and 3 revealed that increment of the antioxidant activity in wine is mainly attributed to the polyphenolic compounds of WRE, with gallic acid being among the major components [3]. Notably, the above results showed that WRE played a similar role to

phenolic compounds in increasing the antioxidant activities in wines, and the effect was particularly significant in white wine, which has a low polyphenolic content. The majority of phenolic compounds is known to exist in the grape skin [12], and hence the low antioxidant activity in white wine would be due to the absence of grape skins in the juice used for wine fermentation [13].

Chromaticity Analysis and Sensory Evaluation

Chromaticity and sensory analyses were carried out for the WRE-added wines to investigate the consumer's preference of their color and flavor (Tables 1 and 2). As shown in Table 1, addition of 0.25% WRE resulted in decreasing redness (a) of red wine, but the same result was not observed in the wine where WRE was added after the completion of fermentation (14 days). Interestingly, addition of WRE after fermentation of white wine resulted in a contradictory change, wherein the yellowness (b) increased, owing to the yellow color of WRE. This result showed that addition of WRE influences the color of wines, especially white wine. In addition, the sensory test results (Table 2)

Table 1. Chromaticity test results of red and white wines containing white rose petal extract (WRE; 0%, 0.10%, or 0.25%).

WRE concentration	Red wine			White wine			
	<i>L</i>	<i>a</i>	<i>b</i>	<i>L</i>	<i>a</i>	<i>b</i>	
Pre-brewing	0%	23.6 ± 0.41 ^a	3.3 ± 0.16 ^a	0.1 ± 0.15 ^a	28.1 ± 0.04 ^a	-0.3 ± 0.01 ^a	-0.1 ± 0.05 ^a
	0.10%	23.3 ± 0.28 ^a	3.1 ± 0.31 ^{ab}	0.1 ± 0.08 ^a	28.1 ± 0.02 ^a	-0.4 ± 0.01 ^a	0.1 ± 0.05 ^a
	0.25%	23.0 ± 0.04 ^a	2.6 ± 0.09 ^b	0.1 ± 0.02 ^a	28.1 ± 0.02 ^a	-0.4 ± 0.04 ^a	0.1 ± 0.15 ^a
Post-brewing	0.10%	23.5 ± 0.22 ^a	3.2 ± 0.26 ^a	0.1 ± 0.05 ^a	28.1 ± 0.04 ^a	-0.4 ± 0.06 ^a	0.4 ± 0.26 ^b
	0.25%	23.3 ± 0.15 ^a	3.1 ± 0.26 ^{ab}	0.1 ± 0.06 ^a	28.2 ± 0.03 ^a	-0.4 ± 0.02 ^a	0.6 ± 0.11 ^b

Means ± standard deviation. In each column, the same superscript letters indicate significant difference ($p < 0.05$). Pre-brewing denotes the wine fermented after addition of WRE, and post-brewing denotes the wine in which WRE was added after the completion of fermentation (15 days).

Table 2. Scores of sensory evaluation of red and white wines containing white rose petal extract (WRE; 0%, 0.10%, or 0.25%).

Sensory evaluation	Red wine			White wine		
	0%	0.10%	0.25%	0%	0.10%	0.25%
Color	5.5 ± 1.37 ^a	5.8 ± 1.38 ^a	5.9 ± 1.88 ^a	5.7 ± 1.43 ^a	5.1 ± 1.53 ^a	4.2 ± 1.95 ^b
Aroma	5.9 ± 1.43 ^a	5.6 ± 1.61 ^a	5.6 ± 1.47 ^a	6.3 ± 1.42 ^a	5.9 ± 1.10 ^a	5.9 ± 1.56 ^a
Astringency	4.8 ± 1.59 ^a	4.9 ± 1.56 ^a	5.3 ± 1.70 ^a	4.9 ± 1.72 ^a	4.8 ± 1.42 ^a	5.0 ± 1.34 ^a
Sweetness	4.3 ± 1.70 ^a	4.2 ± 1.70 ^a	4.4 ± 1.42 ^a	4.2 ± 1.49 ^a	4.5 ± 1.69 ^a	4.7 ± 1.54 ^a
Sourness	4.3 ± 2.15 ^a	4.4 ± 2.02 ^a	4.7 ± 1.91 ^a	4.4 ± 1.81 ^a	4.4 ± 1.59 ^a	4.5 ± 1.84 ^a
Total acceptability	4.6 ± 2.20 ^a	5.1 ± 1.92 ^a	5.2 ± 1.75 ^a	4.6 ± 2.07 ^a	4.5 ± 1.80 ^a	4.6 ± 1.84 ^a

Means ± standard deviation. In each column, the same superscript letters indicate significant difference ($p < 0.05$).

showed that the panel group was not able to distinguish differences in the wine parameters, such as their aroma, sweetness, tartness, astringency, and overall acceptability, except the color of white wine. This result is caused by the color change of white wine observed in the chromaticity test (Table 1). In other words, the sensory test results clearly showed that the overall flavor quality of wines with addition of 0.1% or 0.25% WRE was acceptable to the panels, with slight recognition of color change in the white wine.

White rose petals contain a high concentration of phenolic compounds and are recognized as edible substances. WRE is known to exhibit beneficial health effects, such as antioxidant, anti-microorganism, anti-allergy, and skin-whitening properties. In this study, WRE was prepared by extraction from white rose petals followed by drying. Subsequent brewing of red and white wines was undertaken after addition of 0.1% or 0.25% WRE. The effects of WRE addition were then analyzed by comparing fermentation profiles, colors, sensory test results, and antioxidant activities. It was determined that the addition of WRE did not significantly affect the fermentation profiles of pH, CO₂ production rate, and final ethanol concentration, except for the slow consumption of sugars observed for 0.25% WRE-added white wine. In addition, a slight change in the yellowness of WRE-added white wine was observed, but

the panel responded with the same overall acceptability for the color and aroma qualities of WRE-added wines. However, we noticed that the total polyphenol concentrations increased remarkably in WRE-added wines in a dose-dependent manner, thereby increasing the antioxidant activities of the wines. In summary, we could enhance the beneficial activity of red or white wines by simple addition of WRE without altering the quality characteristics significantly. Considering the additional health effects of WRE such as its anti-*Helicobacter*, anti-allergic, and skin-whitening properties, it could be used as an additive in the production of various beverages or foods.

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