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Physiological Functionalities of *Vitis hybrid* (Sheridan)-*Rubus coreanus* Red Wine Made by *Saccharomyces cerevisiae*

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***Vitis hybrid* (Sheridan)-*Robus coreanus* red wine was vinified by fermentation of a mixture of *Vitis hybrid* and *Robus coreanus* must at 25°C for 10 days. The *Vitis hybrid-Robus coreanus* red wine had ethanol contents of 10.9%. It had high antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity of 57.8% and antioxidant activity of 64.8%. Changes in the physicochemical properties and functionality of the *Vitis hybrid-Robus coreanus* red wine was investigated during a post-fermentation period of three months. The ACE inhibitory activity of the red wine increased as the post-fermentation period prolonged, and showed the highest ACE inhibitory activity of 70.4% 60 days post-fermentation. However, the antioxidant activity declined significantly to 47.2% during the post-fermentation period of 60 days. In terms of sensory evaluation, the *Vitis hybrid-Robus coreanus* red wine had the best acceptability 60 days post-fermentation.**

KEYWORDS : Functionalities, *Saccharomyces cerevisiae* KCTC 7904, *Vitis hybrid* (Sheridan)-*Rubus coreanus* red wine

Grapes contain phenolic compounds which have numerous biological health benefits, such as antioxidant activity [1], inhibitory activity on lipoprotein oxidation [2-4], platelet aggregation inhibitory activity [5], anti-inflammatory activity [6], blood cholesterol lowering activity, and antimicrobial activity [7, 8]. Many results on the health benefits of red wine have also been reported [9-11]. Kallithraka *et al*. [12] reported that red wine may reduce mortality rate from coronary heart disease. Arnous *et al*. [13] also reported that the 1,1-diphenyl-2-picryhydrazyl radical scavenging activity and the hydroxyl radical scavenging activity of red wine were closely correlated. However, only some studies on the quality and acceptability of Korean red wines from various grape varieties have been reported because they are extremely limited in offering unique characteristics, acceptability, and high-value physiological functionality [14, 15]. In spite of some functionalities of Korean red wines, such as the antioxidant activity of wines [16, 17] and the cardiovascular and antidementia functionalities of red wines [18], it is necessary to develop new functional red wines with good acceptability.

Rubus coreanus (Bokbunja) belongs to the family *Rosaceae* and it is found exclusively in South Korea, China, and Japan [19]. It has some therapeutic effects on spermatorrhea, enuresis, asthma, and allergies because it contains various bioactive compounds, including phenolic acids, triterpenosides, flavonoids, and ellagitannin [20]. Therefore, consumption of *Rubus coreanus* has increased as by raw material of some juices and functional foods.

In a previous paper [21], we reported on the quality and antihypertension activity of *Vitis hybrid-Vitis coignetiae* red wine. In this study, we describe physiological functionalities of *Vitis hybrid* (Sheridan)-*Rubus coreanus* red wine brewed with *Saccharomyces cerevisiae* KCTC 7904. We also studied changes in the physicochemical properties and functionality of the red wine during three months of post-fermentation at 4°C.

Materials and Methods

Materials, yeast and chemicals. *Vitis hybrid* (Sheridan) and *Rubus coreanus*, harvested in 2010, were purchased from a commercial market. *Saccharomyces cerevisiae* KCTC 7904 from the Laboratory of Food Biotechnology at Paichai University (Daejeon, Korea) was used for preparing the red wine.

The angiotensin I-converting enzyme (ACE) was extracted from rabbit lung acetone powder (Sigma Chemical Co., St. Louis, MO, USA), and hippuric acid-histidine-leucine, fibrin, pyrogallol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. Unless otherwise specified, all the chemicals were of ana-

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lytical grade.

Vinification of *Vitis hybrid* (Sheridan)-*Rubus coreanus* red wine. *Vitis hybrid* grapes were washed, crushed, and supplemented with *Rubus coreanus* (5%). The mixture was then adjusted to 24° brix by the addition of sugar. After adding 150 ppm of $K_2S_2O_5$, we left the mixture to settle for 5 hr and then inoculated it with 1% *Saccharomyces cerevisiae* KCTC 7904 which was incubated in must for 24 hr. The mixed must was fermented for 10 days at 25°C and then filtered and stored at 4°C for 90 days [18].

General analysis and sensory evaluation. pH was measured with a pH meter (Fisher Scientific, Denver, CO, USA) and the ethanol content was determined with an alcoholic meter (Ceti Optical Instruments, Antwerp, Belgium) after water distillation [22].

Sensory evaluation of the *Vitis hybrid-Rubus coreanus* red wine was performed by 50 trained sensory panels on the basis of a quantitative descriptive analysis [22]. The taste and odor of the red wines were evaluated on a scale of 1 to 5, where 5 was the best score. The mean scores were obtained and plotted as a polygonal graph. The overall acceptability according to the taste and odor was evaluated using the mean value of a hedonic scale with scoring values from 1 (extremely disliked) to 9 (extremely well liked).

Assay of physiological functionalities. After concentrating 50 mL samples of the *Vitis hybrid-Rubus coreanus* red wine to 5 mL, we assayed the activity of ACE inhibition using the method of Cushman and Cheung [23]. We preincubated a mixture containing 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 3 units of ACE, and an appropriate amount of red wine for 10 min at 37°C. The reactions were initiated by the addition of 50 μL of hip-his-leu at a final concentration of 5 mM. The reactions were terminated after 30 min of incubation through the addition of 250 mL of 1.0 N HCl. The liberated hippuric acids were extracted with 1 mL of ethyl acetate and 0.8 mL of the extracts were dried with a Speed Vac Concentrator (EYELA Co., Tokyo, Japan). The residue was then dissolved in 1 mL of the sodium borate buffer and the absorbance was measured at 228 nm to estimate the ACE inhibitory activity.

Fibrinolytic activity was assayed by the method of Fayek and El-Sayed [24]. We added 0.5 mL of each sample to 3 mL of the substrate solution (0.6% fibrin in 0.1 M McIlvaine buffer, pH 7.0) and incubated them at 40°C for 10 min. The reaction was stopped by the addition of 3 mL of 0.4 M TCA for 30 min and then filtered with Whatman filter paper No. 2. We then placed a reaction mixture of 1 mL filtrate, 5 mL 0.4 M Na_2CO_3, and 1 mL 1 N Folin reagent at room temperature for 30 min. The amount of tyrosine released from the fibrin was determined from a tyrosine standard curve based on absorbance measurements at 660 nm. One unit of activity was defined as the production of 1 μg of tyrosine per min for a 1 mL sample.

The 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitory activity was assayed spectrophotometrically by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADPH [25]. A 0.5 mL volume of the reaction mixture contained the following: a potassium phosphate buffer, pH 7.0, 50 μM; dithiothiothreitol, 2 μM; NADPH, 0.3 μM; HMG-CoA, 0.15 μM; and enzyme, 100 μg of protein. Two reaction mixtures were preincubated in a 2 mm light path glass cuvette for 5 min at 37°C. For the assay, we added HMG-CoA to one reaction mixture and we added HMG-CoA with each red wine extract in the other reaction mixture. The mixtures were assayed at 37°C in a recording spectrophotometer. The initial velocity of the reaction was measured and the net rate of the NADPH oxidation was determined by subtracting the rate of oxidation in the absence of HMG-CoA from the rate observed with both substrates present. We calculated the HMG-CoA reductase inhibitory activity as follows:

$$\text{HMG-CoA reducatse inhibitory activity (\%)} = [1 - (A_{340} \text{ of sample} - A_{340} \text{ sample of blank})/(A_{340} \text{ of control} - A_{340} \text{ control of blank})] \times 100$$

The antioxidant activity was assayed using DPPH [17]. A 0.8 mL DPPH solution (12.5 mg of DPPH dissolved in 100 mL of ethanol) was added to 0.2 mL of a sample, shaken for 10 sec, and left for 10 min. We then determined the absorbance at 525 nm. The antioxidant activity was calculated as [1 – (absorbance of reaction mixture-absorbance of sample alone)/absorbance of blank] × 100.

Results and Discussion

Ethanol content and physiological functionalities of *Vitis hybrid* (Sheridan)-*Rubus coreanus* red wine. As shown in Table 1, ethanol content of the *Vitis hybrid-Rubus coreanus* red wine was 10.9% after fermentation for 10 days. This was similar or slightly lower than results of a previous study on *Vitis hybrid-Vitis coignetiae* red wine (13.9%) [21] and than the alcohol content in four kinds of Korean red wines (11.4 to 12.0%) [18].

Vitis hybrid-Rubus coreanus red wine had antihypertensive ACE inhibitory activity of 57.8% after fermentation for 10 days. This ACE inhibitory activity of the red wine was lower than the corresponding value of *Vitis hybrid-Vitis coignetiae* red wine (68.5%) [21], *Paecilomyces japonica* wine (67.3%) [26], *Ganoderma lucidum* wine (63.4%) [22], Korean *Vitis labrusca* L (Concord) red wines (65.1%) [18], and chamomile wine (36.7%) [27].

Table 1. Ethanol content and physiological functionalities of *Vitis hybrid* (Sheridan)-*Robus coreanus* red wine after 10 days of fermentation

Ethanol (%)	Angiotensin I-converting enzyme inhibitory activity (%)	Fibrinolytic activity (clean zone: mm)	HMG-CoA reductase inhibitory activity (%)	Antioxidant activity (%)
10.9	57.8	2.5	n.d	64.8

HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; n.d, not detected.

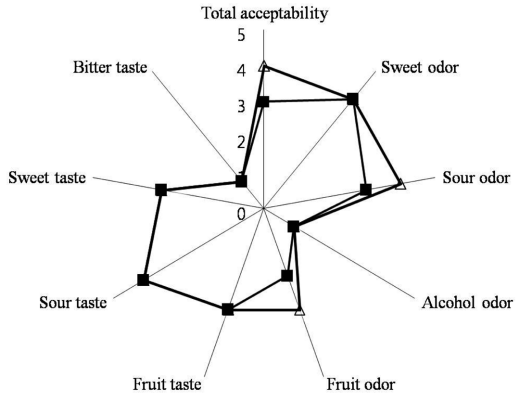
Table 2. Changes of ethanol content, angiotensin-converting enzyme (ACE) inhibitory activity, and antioxidant activity of *Vitis hybrid-Robus coreanus* red wine during post-fermentation

Components	Post-fermentation periods (days)	*Vitis hybrid-Robus coreanus* red wine
pH	0	3.74
	30	3.70
	60	3.70
	90	3.70
Ethanol (%)	0	11.6
	30	10.4
	60	10.2
	90	10.4
ACE inhibitory activity (%)	0	57.8
	30	66.2
	60	70.4
	90	67.9
Antioxidant activity (%)	0	64.8
	30	60.5
	60	47.2
	90	47.5

Furthermore, *Vitis hybrid-Rubus coreanus* red wines had an antioxidant activity of 64.8% after 10 days fermentation. This suggested that consumption of this red wine may help in regulating free radical reaction-mediated disorders, such as coronary heart disease, atherogenesis, and aging. No fibrinolytic activity or HMG-CoA reductase inhibitory activity of *Vitis hybrid-Rubus coreanus* red wine were detected or were detected in only very low levels.

Changes in the antihypertensive ACE inhibitory activity and antioxidant activity of *Vitis hybrid-Rubus coreanus* red wine during post-fermentation. As shown in Table 2, the ACE inhibitory activity of the *Vitis hybrid-Rubus coreanus* red wine increased as the post-fermentation period extended to 60 days. After 60 days of post-fermentation, the level of ACE inhibitory activity reached 70.4%. However, the antioxidant activity was significantly decreased to 47.2% during a post-fermentation period of 60 days.

Sensory characteristics of the *Vitis hybrid-Rubus coreanus* red wine. The total acceptability of the *Vitis hybrid-Rubus coreanus* red wine after 60 days and 90 days of post-fermentation was investigated (Fig. 1). The *Vitis hybrid-Rubus coreanus* red wine had a strong sour and sweet flavors and very weak bitter or alcoholic flavors. From this sensory evaluation, we concluded that the *Vitis hybrid-Rubus coreanus* red wine after 60 days of post-fermentation is more acceptable than after 90 days of post-fermentation. These results are similar to *Vitis hybrid-Vitis coignetiae* red wine after 60 days of post-fermentation [21].



△, *Vitis hybrid* (Sheridan)-*Robus coreanus* red wine after 60 days of post-fermentation
■, *Vitis hybrid* (Sheridan)-*Robus coreanus* red wine after 90 days of post-fermentation

Fig. 1. The quantitative descriptive analysis profile for taste and odor of *Vitis hybrid* (Sheridan) *Robus coreanus* red wine after 60 days and 90 days of post-fermentation.

In conclusion, the optimal vinification process of the *Vitis hybrid-Rubus coreanus* red wine was done using 5% *Rubus coreanus* in *Vitis hybrid* must. The mixture was fermented for 10 days at 25°C with *Saccharomyces cerevisiae* KCTC 7904 and then subjected to 60 days of post-fermentation. We suggest that the *Vitis hybrid-Rubus coreanus* red wine from this study is a new functional red wine with a high antioxidant activity, high antihypertension, and good acceptability.

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