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Effects of *Vitis coignetiae* on the Quality and Antihypertension of *Vitis hybrid* Red Wine

Jang, Jeong-Hoon[1], Sung-Hun Yi[2], Jae-Ho Kim[2], Dae-Hyoung Lee[3], and Jong-Soo Lee[1]*
[1]Dept. of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735 Korea
[2]Korea Food Research Institute, Seongnam 463-746, Korea
[3]Gyeonggi-do Agricultural Research and Extension Services, Hwasung 449-702, Korea

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The goal of this study was to develop a high value Korean red wine possessing antihypertension activity. The effects of some medicinal plants and grapes on the alcohol fermentation process and the angiotensin I-converting enzyme (ACE) inhibitory activity of *Vitis hybrid* red wine were investigated. Various *Vitis hybrid* red wines were vinified by the fermentation of a mixture of *Vitis hybrid* must and some medicinal plants and grapes at 25°C for 10 days. Of these red wines, the *Vitis hybrid–Vitis coignetiae* red wine exhibited a high ethanol content of 12.0% and had a good level of acceptability. It also showed a high antihypertensive ACE inhibitory activity of 68.5%. After post-fermentation of 60 days, the ACE inhibitory activities of the *Vitis hybrid–Vitis coignetiae* red wine exhibited the highest ACE inhibitory activity of 80.7% (IC_{50}: 28 mg/mL) and also had the best acceptability. The C_{18} solid phase extracts of the *Vitis hybrid–Vitis coignetiae* red wine, after 60 days post-fermentation, showed clear antihypertensive effects on spontaneously hypertensive rats. Our results reveal that the *Vitis hybrid–Vitis coignetiae* red wine has the potential to become a new functional red wine due to its good acceptability and high antihypertensive activity.

Key words: Quality, antihypertension, *Vitis hybrid-Vitis coignetiae* red wine

Introduction

Various fruits, medicinal plants and herbs have recently received attention because they contain nutraceuticals with health-stimulating properties. Grapes in particular contain 0.3% to 0.5% of various organic acids; they also have some free sugars and a large amount of polyphenol compounds such as flavonoids containing anthocyanin and proanthocyanidins [5, 30], and phenolic acids [27]. It has recently come to light that phenolic compounds have numerous biological health benefits such as the following: antioxidant activity [8] due to the scavenging of active harmful oxygen radicals [5, 7, 25, 29] inhibition of oxidation on lipoprotein [26, 27] low density lipoprotein [17, 28] platelet aggregation inhibition [2] anti-inflammatory action [18] the lowering of blood cholesterol by the resveratrol of grapes; and antimicrobial activity [15].

Many research results on the health benefits of red wine have been reported [5, 23]. Kimura *et al*. [11] and Kinsella *et al*. [12] reported that red wine may reduce the mortality rate from coronary heart disease. Furthermore, some studies have focused on the vinification of various korean grape varieties and reported on their quality and acceptability [13, 16], and the antioxidant activity of wines [3, 13] and the cardiovascular and antidementia functionalities of red wines [20]. Korean red wine, however, has not been developed with excellent acceptability and high-value physiological functionality.

In this study, for the improvement of acceptability and antihypertension of *Vitis hybrid* red wine by addition of medicinal plants, we selected grapes (Campbell Early, wild grape) and medicinal plants (*Robus coreanus*, Tea plant, Gugija, Mulberry) which were known its some functionality and good flavor and taste [9, 21, 22]. Effect of medicinal plants and grapes on the quality and antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity

*Corresponding author
Tel: +82-42-520-5388, Fax: +82-42-520-5388
E-mail: biotech8@pcu.ac.kr

of new *Vitis hybrid* (Sheridan) red wine were investigated.

Materials and Methods

Materials and Chemicals

Various medicinal plants and grapes harvested in 2010, namely *Rubus coreanus*, *Camellia sinensis* (Tea plant), *Lycium fructus* (Fruits of Gugija), *Morus alba* (Mulberry fruit), *Vitis labruscana B* (Campbell Early), *Vitis coignetiae* (Wild grape) and *Vitis hybrid* (Sheridan), were purchased from the commercial market. *Saccharomyces cerevisiae* KCTC 7904 from the Laboratory of Food Biotechnology at Paichai University (Daejeon, Republic of Korea) was used for preparing the red wines.

The ACE used in this study was extracted overnight from rabbit lung acetone powder (Sigma Chemical Co., St. Louis, USA) using 100 mM sodium borate buffer (pH 8.3) containing 300 mM NaCl at 4°C, and hippuric acid-histidine-leucine were also purchased from Sigma Chemical (St. Louise, MO). Unless otherwise specified, all the chemicals were of analytical grade.

Vinification of Various *Vitis hybrid* (Sheridan) Red Wines

Vitis hybrid was crushed, and filtered to prepare juice, and then supplemented with various medicinal plants and grapes (10%, w/v), except *Rubus coreanus* (5%, w/v). The mixture was again crushed and 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 h and then inoculated with 1% *Saccharomyces cerevisiae* KCTC 7904 which was incubated in must for 24 h. The complex musts were fermented for 10 days at 25°C and filtered and then stored at 4°C for 90 days as post-fermentation [24].

General Analysis and Sensory Evaluation

The pH values were measured with a pH meter (Fisher Scientific, Colorado, USA). The titratable acidity was estimated after titration with 0.1 N NaOH to pH 7.0 and calculated the tartaric acid percentage from this value. The ethanol content was determined with an alcoholic meter (Ceti Optical Instruments, Belgium) after water distillation [10]. After the distillation of red wines, we determined the volatile acid in terms of titratable acidity. The reducing sugar content was determined according to the DNS method.

Total anthocyanin and phenol content were determined by the method of Morris *et al*. [19]. The color of the red wines were determined by using Hunter colorimeter (Hunter Associates Laboratories, Reston, USA) and ascribed the values of L (for lightness-darkness), a (for redness-greenness), and b (for yellowness-blueness).

The sensory evaluation of the *Vitis hybrid* red wines was estimated by 10 experienced tasters on the basis of a quantitative descriptive analysis [10]. 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 by using the mean value of a hedonic scale with scoring values from 1 (extremely disliked) to 9 (extremely well liked).

Assay of ACE Inhibitory Activity

The ACE inhibitory activity was assayed according to a modified method of Cushman and Cheung [4]. A mixture containing 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 3 units of ACE, and 50 μL of sample (1 mg of the freeze-dried extracts was dissolved in 50 μL of 100 mM sodium borate buffer, pH 8.3) was preincubated for 10 min at 37°C. The reactions was initiated by the adding 50 μL of hippuric-histidine-leucine at a final concentration of 5 mM and terminated after 30 min of incubation by adding 250 mL of 1.0 N HCl. The hippuric acids liberated was extracted with 1 mL of ethyl acetate, and 0.8 mL of the extracts was evaporated until dry using a Speed Vac Concentrator (EYELA, Tokyo, Japan). The residue was then dissolved in 1 mL of the sodium borate buffer, and the absorbance at 228 nm was measured to estimate the ACE inhibitory activity. The inhibition activity was calculated using the following equation.

$$\text{Inhibition activity } (\%) = [1 - (A\text{-}B)/(C\text{-}D)] \times 100$$

Where A is the absorbance of the solution containing ACE, substrate and sample, B is the absorbance of the solution containing ACE and sample without the substrate, C is the absorbance of the solution containing ACE and substrate without the sample, D is the absorbance of the solution containing only substrate.

C_{18} Solid Phase Extraction

ACE inhibitor of *Vitis hybrid-Vitis coignetiae* red wine was partially purified by C_{18} solid phase extraction chromatography as follows. 50 mL of the concentrated *Vitis hybrid-Vitis coignetiae* red wine was applied to C_{18} solid phase extraction (Sep-Pak C_{18} Cartridges, Waters Co., Milford, MA, USA), equilibrated with 5% acetonitrile. A gradient was carried out with 5, 50 and 100% acetonitrile. The active fraction of 50% acetonitrile was collected and lyophilized immediately and then it used in the orally administration of spontaneously hypertensive rat.

Antihypertension Action of the Red Wine

Spontaneously hypertensive male rats (SHR, SHR/NCrljOri) weighing 180 to 200 g and 10 weeks old, were purchased from Orientbio Co., Korea. SHRs were housed individually in steel cage on a room of at 24°C with a 12 h light-dark cycle, and fed standard diet. Tap water was freely available. The C_{18} solid phase extracts of the red wine after 60 days post-fermentation was orally administered to the spontaneous hypertension rat (SHR) at dosage of 500 mg/kg, and then the systolic blood pressure was measured before as well as after 0.5 h, 1 h, 2 h, and 4 h of administration from rat tail using a specially devised Blood Pressure Monitoring System (Kent Scientific, Torrington, CT). Each experimental group consisted of five SHRs, and a negative and a positive control group were also provided: The positive control group was administered a commercial antihypertensive drug, captopril (ACE inhibitor), at a dosage of 100 mg/kg, whereas saline was administered to the negative control group. Prior to the administration of the C_{18} solid phase extract, the blood pressures of the SHRs were measured four times during a one-day period, and the test groups were selected according to their average blood pressure. While the ACE inhibitor was being administered, the blood pressure of each group was measured three times during every test.

Results and Discussion

Manufacture and Characteristics of Various *Vitis hybrid* Red Wines

After the fermentation for the six kinds of *Vitis hybrid* red wines by using *Vitis hybrid* and medicinal plants, we investigated their physicochemical properties (Table 1). The ethanol content of various *Vitis hybrid* red wines ranged from 10.6% to 13.4% after fermentation for 10 days and differed slightly from the results of a previous study on *Vitis hybrid* red wine (11.6%) [20]. Moreover, our results were similar to those of the alcohol content in four kinds of Korean red wines (11.4% to 12.0%) [20] and one type of sweet persimmon wine (12.8%) [3]. The total acid content were higher in all the other red wines than that of the *Vitis hybrid* red wine as control, because various acidic sub-

Table 1. Physicochemical properties of the various *Vitis hybrid* red wines.

Chemical components \ Red wines		*Vitis hybrid* (*Vh*) red wine[1] (control)	*Vh* - *Robus coreanus* red wine	*Vh* - *Camellia sinensis* red wine	*Vh* - *Lycium fructus* red wine	*Vh* - *Mulberry* red wine	*Vh* - *Vitis labruscana B* red wine[2]	*Vh* - *Vitis coignetiae* red wine
Ethanol (%)		11.6	11.6	13.4	11.4	10.6	12.0	13.9
pH		3.82	3.74	3.97	3.97	3.79	3.65	3.70
Total acid (%)		0.78	1.76	1.20	1.20	1.47	1.12	1.11
Volatile acid (%)		0.011	0.011	0.010	0.010	0.009	0.011	0.012
Residual sugar (mg/mL)		7.40	3.84	1.46	3.78	1.17	1.00	0.99
Total anthocyanin (A_{520})		1.37	2.61	1.65	1.77	1.90	1.29	1.91
Color intensity (A_{530}+A_{430})		1.91	5.13	3.74	4.27	3.98	2.75	4.09
Brownning index (A_{520}/A_{420})		0.68	0.96	1.26	1.41	1.09	1.14	1.14
Total phenol (mg/L)		55.23	177.19	163.09	121.30	60.10	52.14	60.50
Hunters color Value	L[3]	77.51	34.16	80.37	82.60	82.32	+1.37	80.36
	a[4]	+16.14	+67.09	+11.41	+3.36	+9.75	+8.12	+10.75
	b[5]	+1.17	+51.93	+11.36	+23.84	+14.95	-19.09	+

[1]*Vitis hybrid* red wines were obtained from fermentation of 10 days after addition of medicinal plants and grapes into *Vitis hybrid* must

[2]*Vitis labruscana B* and *Vitis coignetiae* were Compbell Early and wild grape, respectively

[3]Measure lightness and varies from 100 for perfect white to zero for black

[4]Measure redness when plus, gray when zero and greenness when minus

[5]Measures yellowness when plus and blueness when minus.

stances such as organic acid were extracted from medicinal plants and other grapes. The total phenol content were two or three times higher in *Vitis hybrid-Rubus coreanus*, *Vitis hybrid-Camellia sinensis* and *Vitis hybrid-Lycium fructus* red wines than those of *Vitis hybrid* red wine, because they contained a large amount of phenolic compounds than those of the other plants and it were caused by extraction during fermentation. The total anthocyanin content which are also known as bioactive compound, was the highest in *Vitis hybrid-Rubus coreanus* red wine.

Of the six kinds of *Vitis hybrid* red wines, the *Vitis hybrid–Vitis coignetiae* red wine showed the highest antihypertensive ACE inhibitory activity (68.5%) after fermentation for 10 days (Table 2). As described in Table 1, even though total phenol content were higher in *Vitis hybrid-Rubus coreanus*, *Vitis hybrid-Camellia sinensis* and *Vitis hybrid-Lycium fructus* red wine, ACE inhibitory activity was higher in *Vitis hybrid-Vitis coignetiae* red wind rather than those of these red wine. Meanwhile, this ACE inhibitory activity of the *Vitis hybrid-Vitis coignetiae* red wine was similar or higher than the corresponding value of the following: *Paecilomyces japonica* wine (67.3%) [15], *Ganoderma lucidum* wine (63.4%) [10], Korean *Vitis labrusca L* (Concord) red wines (65.1%) [20], and chamomile wine (36.7%) [14].

The total acceptability of the *Vitis hybrid* red wines were investigated. The *Vitis hybrid-Vitis coignetiae* and *Vitis hybrid-Rubus coreanus* red wines show good acceptability (data not shown). On the basis of the alcohol content, as well as the antihypertension and acceptability, we assessed the *Vitis hybrid-Vitis coignetiae* red wine to be highly valuable red wine.

Changes of Quality and ACE Inhibitory Activity of the *Vitis hybrid-Vitis coignetiae* Red Wine During Post-Fermentation

We investigated changes of the ethanol content, total anthocyanin and phenol, and antihypertensive ACE inhibitory activity of *Vitis hybrid-Vitis coignetiae* red wine during post-fermentation. As shown in Table 3, ethanol content, total anthocyanin and phenol content were slightly decreased. However, the ACE inhibitory activity of the *Vitis hybrid-Vitis coignetiae* red wine increased as the post-fermentation period was extended to 60 days. After 60 days of post-fermentation, the level of ACE inhibitory activity reached 80.7% (IC_{50}: 28 mg/mL) in the red wine. Therefore, further study is going on bioactive compounds which were affected in its ACE inhibitory activity during fermentation and post-fermentation of the *Vitis hybrid-Vitis coignetiae* red wine.

The total acceptability of the *Vitis hybrid-Vitis coignetiae* red wine after 60 days and 90 days of post-fermentation was investigated (Fig. 1). The *Vitis hybrid-Vitis coignetiae*

Table 3. Changes of ethanol, total anthocyanin and phenol, and angiotensin I-converting enzyme (ACE) inhibitory activity in the *Vitis hybrid–Vitis coignetiae* red wine during post-fermentation.

Components	Post-fermentation periods (days)	*Vitis hybrid-Vitis coignetiae* red wine
Ethanol (%)	0	12.0
	30	10.4
	60	10.8
	90	10.6
Total anthocyanin (A_{520})	0	1.91
	30	1.26
	60	1.31
	90	1.03
Total phenol (mg/L)	0	60.50
	30	52.11
	60	46.69
	90	39.97
ACE inhibitory activity (%)	0	68.5
	30	72.2
	60	80.7
	90	73.5

Table 2. Angiotensin I-converting enzyme (ACE) inhibitory activity of the various *Vitis hybrid* red wines.

Functionalities \ Red wines	*Vitis hybrid* (Vh) red wine[1] (contol)	*Vh - Robus coreanus* red wine	*Vh - Camellia sinensis* red wine	*Vh - Lycium fructus* red wine	*Vh - Mulberry* red wine	*Vh - Vitis labruscana B* red wine[2]	*Vh - Vitis coignetiae* red wine
ACE inhibitory activity (%)	53.8(±0.8)	58.9(±0.2)	n.d[3]	63.2(±1.1)	63.1(±0.9)	60.5(±0.8)	68.5(±0.7)

[1] *Vitis hybrid* red wines were obtained from fermentation of 10 days after addition of medicinal plants and grapes into *Vitis hybrid* must.
[2] *Vitis labruscana B* and *Vitis coignetiae* were Campbell Early and wild grape, respectively.
[3] n.d: not detected.

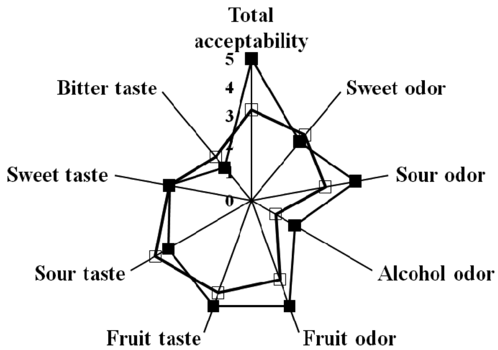

Fig. 1. The quantitative descriptive analysis profile for taste and odor of *Vitis hybrid-Vitis coignetiae* red wine after 60 days and 90 days of post-fermentation. ■, *Vitis hybrid* (Sheridan)-*Vitis coignetia*e (Wild type) red wine after 60 days of post-fermentation; □, *Vitis hybrid* (Sheridan)-*Vitis coignetiae* (Wild type) red wine after 90 days of post-fermentation.

red wine had a strong sour taste and fruity flavor but showed very weak levels and no significant difference in terms of sweetness, bitterness or alcoholic flavor. From this sensory evaluation, we concluded that the *Vitis hybrid-Vitis coignetiae* red wine has the best acceptability after 60 days of post-fermentation. The color of the *Vitis hybrid-Vitis coignetiae* red wine was also evaluated as good (bright reddish-pink). No *et al*. [20] reported that *Vitis hybrid* red wine had a higher level of acceptability than *Vitis labrusca*, *Vitis labrusca B*, and *Vitis labrusca L* (Concord) red wines; in addition, Lee *et al*. [16] reported that the *Vitis labrusca B* and *Vitis labrusca* varieties were suitable for making red wine.

In conclusion, optimal vinification process of the *Vitis hybrid-Vitis coignetiae* red wine was that 10% *Vitis coignetiae* was added to the *Vitis hybrid* must and fermented for 10 days at 25°C with *Saccharomyces cerevisiae* and then the wine was subjected to 60 days of post-fermentation. We suggests that the *Vitis hybrid–Vitis coignetiae* red wine has the potential to become a new functional red wine with a high antihypertensive property and good acceptability.

Antihypertensive Action of the *Vitis hybrid-Vitis coignetiae* Red Wine

We investigated antihypertensive action of the C_{18} solid phase extract from the red wine after 60 days post-fermentation by using SHR. As shown in Fig. 2, the average blood pressure of the SHRs in the ACE inhibitor group was about 180 mmHg just before administration of the ACE inhibitor. An half hour after administration the ACE inhibitor at a dosage of 500 mg/kg rat, the blood pressure measured decreased to 140 mmHg, similar with the commercial captopril; and the average blood pressure then ascended quickly rather than commercial antihypertensive drug, captopril. This suggests that the partial purified ACE inhibitor produce a clear antihypertensive effect in SHR at a dosage of 500 mg/kg.

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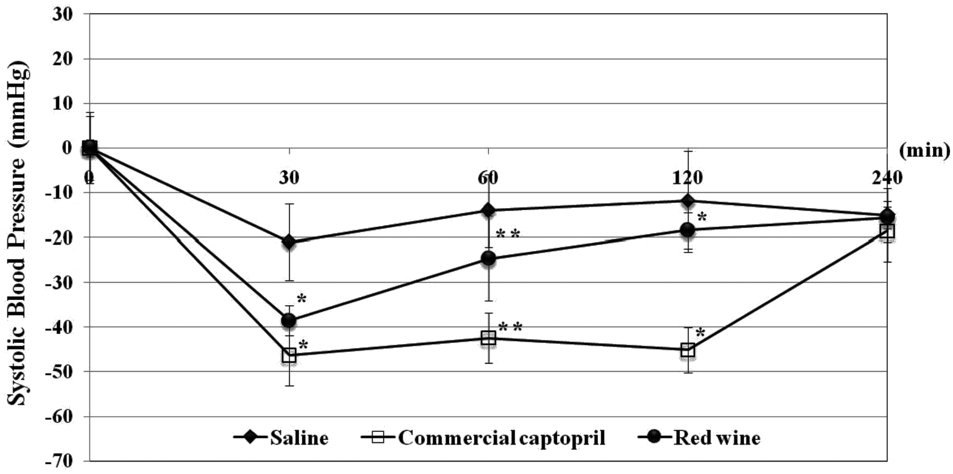

Fig. 2. Effect of orally administered the C_{18} solid phase extract of *Vitis hybrid–Vitis coignetiae* red wine after 60 days post-fermentation on blood pressure in SHR.

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국문초록

쉐리단 포도주의 항고혈압성과 품질에 머루첨가의 영향

장정훈[1] · 이성훈[2] · 김재호[2] · 이대형[3] · 이종수[1]*
[1]배재대학교 생명유전공학과, [2]한국식품연구원, [3]경기도 농업진흥청

항고혈압활성을 가진 고품질 국산 포도주를 개발하기 위하여 쉐리단 포도주 제조과정에서 다양한 약용식물과 머루 등을 첨가하여 이들이 쉐리단 포도주의 품질과 생리기능성에 미치는 영향을 조사하였다. 쉐리단 포도 주스에 머루를 첨가하여 발효시킨 쉐리단-머루 포도주가 에탄올 함량과 기호도가 가장 높았고 68.5%의 높은 항고혈압성 안지오텐신 전환효소(ACE) 저해활성을 보였다. 쉐리단-머루 포도주는 60일간 후발효 시켰을 때 ACE 저해활성은 80.7%(IC_{50}: 28 mg/mL)로 상승하였고 기호성도 좋았다. 또한 60일 후발효 시킨 쉐리단-머루 포도주의 C_{18} solid phase 추출물은 본태성 고혈압쥐 (SHR)에서 항고혈압성이 확인되었다.