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Antihypertensive Angiotensin I-Converting Enzyme Inhibitory Activity and Antioxidant Activity of *Vitis* hybrid-*Vitis coignetiae* Red Wine Made with *Saccharomyces cerevisiae*

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A *Vitis* hybrid-*Vitis coignetiae* red wine was vinified by fermentation of a mixture of a *Vitis* hybrid–*Vitis coignetiae* must with *Saccharomyces cerevisiae* KCTC 7904 at 25°C for 10 days. The *Vitis* hybrid-*Vitis coignetiae* red wine showed high antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity (67.8%) and antioxidant activity (76.7%). The antihypertensive ACE inhibitor in the *Vitis* hybrid-*Vitis coignetiae* red wine was partially purified by solid phase extraction chromatography, and its ACE inhibitory activity yielded an IC_{50} of 1.8 mg/mL. Six kinds of oligopeptides, including five new kinds, were contained in the partially purified ACE inhibitor fraction from the red wine after 10 days of fermentation. Antioxidant activity decreased significantly from 76.7% to 40.5% when the post-fermentation period was prolonged to 30 days.

KEYWORDS : Antihypertensive ACE inhibitory activity, Antioxidant activity, *Vitis* hybrid-*Vitis coignetiae* red wine

Many studies have reported the health benefits of red wine [1-6]; however, only a few have investigated the cardiovascular and anti-dementia functionalities of red wines [7]. In a previous study [8], the effect of *Vitis coignetiae* on the quality and anti-hypertensive effects of a new *Vitis* hybrid (Sheridan) red wine were investigated. In this study, the angiotensin I-converting enzyme (ACE) inhibitory and antioxidant activities of *Vitis* hybrid-*Vitis coignetiae* red wine were investigated.

V. coignetiae (Wild grape) and *Vitis* hybrid (Sheridan) grapes 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 wines. The ACE used in this study was extracted overnight from rabbit lung acetone powder (Sigma Chemical Co., St. Louis, MO, USA) using 100 mM sodium borate buffer (pH 8.3) containing 300 mM NaCl at 4°C. Hippuric acid-histidine-leucine and DPPH were also purchased from Sigma Chemical. Unless otherwise specified, all chemicals were of analytical grade.

The *Vitis* hybrid grapes were crushed, filtered to prepare juice, and then supplemented with *V. coignetiae* (10%, w/v). The mixture was again crushed and adjusted to 24° brix by adding sugar. After adding 150 ppm $K_2S_2O_5$, the mixture was left to settle for 5 hr and then inoculated with 1% *S. cerevisiae* KCTC 7904, which had been incubated in must for 24 hr. The complex musts were fermented for 10 days at 25°C and filtered [9].

ACE inhibitory activity was assayed according to a modified method of Cushman and Cheung [10]. A mixture containing 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 3 units ACE, and 50 μL 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 reaction was initiated by adding 50 μL hippuric-histidine-leucine at a final concentration of 5 mM and terminated after 30 min of incubation by adding 250 mL 1.0 N HCl. The hippuric acid liberated was extracted with 1 mL of ethyl acetate, and 0.8 mL of the extract was evaporated to dryness using a Speed Vac Concentrator (EYELA, Tokyo, Japan). The residue was then dissolved in 1 mL sodium borate buffer, and the absorbance was measured at 228 nm to estimate ACE inhibitory activity using the following equation:

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

where A is the absorbance of the solution containing the ACE, substrate, and sample; B is the absorbance of the solution containing ACE and the sample without the substrate; C is the absorbance of the solution containing ACE and the substrate without the sample; and D is the absor-

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Table 1. Physiological functionality of *Vitis* hybrid (Sheridan)-*Vitis coignetiae* red wine

Angiotensin I-converting enzyme inhibitory activity (%)	Fibrinolytic activity (clean zone: mm)	HMG-CoA reductase inhibitory activity (%)	Antioxidant activity (%)
67.8	1.0	n.d.	76.7

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

bance of the solution containing only the substrate.

Fibrinolytic activity was assayed as follows [11]. We added 0.5 mL of each sample to 3 mL of the substrate solution (0.6% fibrin in 0.1 M McIlvaine's buffer, pH 7.0) and incubated them at 40°C for 10 min. The reaction was stopped by adding 3 mL 0.4 M trichloriacetic acid for 30 min and the mixture was filtered with Whatman No. 2 filter paper (Whatman International Ltd., Clifton, NJ, USA). We then placed a reaction mixture of 1 mL of filtrates, 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 minute for a 1 mL sample.

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 [11]. A 0.5 mL volume of the reaction mixture contained the following: 50 μM potassium phosphate buffer, pH 7.0, 2 μM dithiothreitol, 0.3 μM NADPH, 0.15 μM HMG-CoA, and 100 μg of protein (enzyme). Two reaction mixture aliquots were preincubated in 2 mm light-path glass cuvettes for 5 min at 37°C. For the assay, we added HMG-CoA to one reaction mixture aliquots and added HMG-CoA with each red wine extract to the other reaction mixture aliquot. The aliquots were assayed at 37°C in a recording spectrophotometer. The initial velocity of the reaction was measured, and the net rate of NADPH oxidation was determined by subtracting the rate of oxidation in the absence of HMG-CoA from the rate observed when both substrates were present. Thus, we calculated the HMG-CoA reductase inhibitory activity as follows:

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

Antioxidant activity was assayed as follows using DPPH [11]. A 0.8 mL DPPH solution (12.5 mg DPPH dissolved in 100 mL ethanol) was added to 0.2 mL of 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.

The ACE inhibitor contained in the *Vitis* hybrid-*Vitis coignetiae* red wine was partially purified by solid phase extraction chromatography as follows. Fifty mL of concentrated *Vitis* hybrid-*Vitis coignetiae* red wine was applied to a C_{18} solid phase extraction (Sep-Pak C_{18} Cartridge; Waters Co., Milford, MA, USA), and the peptides in the active fraction were analyzed by liquid chromatography-mass spectrometry (MS). The active fractions were separated by 12% SDS-PAGE and in-gel digested, as described previously [12]. The digests were resolved in 15 mL 0.02% formic acid in 0.5% acetic acid. The peptide samples (10 mL) were concentrated on an MGU30-C_{18} trapping column (LC Packings, San Francisco, CA, USA) and subjected to a nanocolumn (10 cm, 75 μm i.d., C_{18} reverse-phase column, Proxeon Biosystems, Staermosegaardsvej, Denmark) at a flow rate of 120 nL/min. Subsequently, the peptides were eluted from the column by applying a gradient of 0~65% acetonitrile for 80 min at the same flow rate. All MS and MS/MS spectra were acquired in data-dependent mode using a LCQ-Deca ESI ion trap mass spectrometer (Thermo Finnigan Co., San Jose, CA, USA). The MS/MS spectra were identified using SEQUEST ver. 3.3 software (Thermo Finnigan Co.).

Functional properties of *Vitis* hybrid (Sheridan)-*V. coignetiae* red wine. After 10 days of fermentation, the *Vitis* hybrid-*V. coignetiae* red wine yielded an ethanol content of 13.5% and 67.8% antihypertensive ACE inhibitory activity (Table 1). Furthermore, the antioxidant activity was 76.7%, but no or very low levels of fibrinolytic and HMG-CoA reductase inhibitory activity were detected.

ACE inhibitory peptides and antioxidant activity. After the partial purification of the ACE inhibitor from the *Vitis* hybrid-*V. coignetiae* red wine by systematic solvent extraction and solid phase extraction chromatography, an active fraction with an IC_{50} of 1.8 mg/mL was obtained. Six kinds of oligopeptides, including five new kinds, were contained in the red wine after a 10 day fermentation (Table 2). Therefore, we chemically synthesized these oligopeptides, and we plan to identify the antihypertensive ACE inhibitory peptides after determining their ACE inhibitory activity. Recently, various ACE inhibitors with antihypertensive effects [13] have been isolated from Korean traditional alcoholic beverages [12] and sake and its by-products [9]. Almost all ACE inhibitors are peptides or a complex of glycoprotein and polyphenol [14], except for triterpene [11]. Many different sequences of ACE inhibitors have been reported, ranging from tripeptides to oligopeptides [9].

Table 2. Peptide analysis of the *Vitis* hybrid-*Vitis coignetiae* red wine after 0 and 10 days of fermentation

Fermentation periods	Peptide sequence
0 days	GKHEAQLGAGAAHVEAL
	YSSSSLN
	QLTEEFWDFVFLGQGSADEVSKKTG
	YVGKTPGTI
	LSGQTIEVTSEYLFR
	ETVPPAVDLVLGASACC
	AGLSFSG
	LQGGFVWDWVDQSLIK
	GLVVAVGAGAKNSNGTF
	DVVRGGAPYGMTTTADGDGSRQPSAQEL
	RTKGPCVVEENDPIFERGST
	VMKGANSYEEIAGADVCIVTAGVPRKP
	CDIMTGLAFLHSQGIVH
	QIGFVLYPLGGAP
	YVGKTPGTI
10 days	LAGNGRSVGVEYV
	ATQVPLVSSTSEGYTASQPLYQP
	NPADLPDAG
	TRHNIGFAAVDALARAWNISLA
	TTTAATS
0 & 10 days	REGAAPGAA

Although the peptide sequences of the ACE inhibitor from this red wine were longer and thus were degraded more easily in the stomach by some proteases and more difficult to absorb in the intestine, this is the first report of unique antihypertensive ACE inhibitory oligopeptides in red wine. In a previous study [8], we demonstrated the antihypertensive action of a partially purified ACE inhibitor using the spontaneous hypertensive rat.

The antioxidant activity of the *Vitis* hybrid-*V. coignetiae* red wine decreased significantly from 76.7% to 40.5% during a 30 day post-fermentation period and remained unchanged thereafter (data not shown), although its ACE inhibitory activity increased about 12% during a 60 day post-fermentation period in a previous study [8].

References

1. Kimura Y, Ohminami H, Okuda H, Baba K, Kozawa M, Arichi S. Effects of stilbene components of roots of polygonum ssp. on liver injury in peroxidized oil-fed rats. Plant Med 1983;49:51-4.
2. Koh KH, Lee JH, Yoon KR, Choi SY, Seo KL. Phenolic compounds of Korean red wine and their superoxide radical scavenging activity. Food Sci Biotechnol 1998;7:131-6.
3. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992;339: 1523-6.
4. Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993;341:454-7.
5. Kinsella JE, Frankel E, German B, Kanner J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol 1993;47:85-9.
6. Choi Y, Yu KW, Han NS, Koh JH, Lee J. Antioxidant activities and antioxidant compounds of commercial red wines. J Korean Soc Food Sci Nutr 2006;35:1286-90.
7. No JD, Lee DH, Hwang YS, Lee SH, Lee DH, Lee JS. Changes of physicochemical properties and antioxidant activities of red wines during fermentation and post-fermentation. Korean J Microbiol Biotechnol 2008;36:67-71.
8. Jang JH, Yi SH, Kim JH, Lee DH, Lee JS. Effects of *Vitis coignetiae* on the quality and antihypertension of *Vitis hybrid* red wine. Korean J Microbiol Biotechnol (in press).
9. Saito Y, Wanezaki K, Kawato A, Imayasu S. Structure and activity of angiotensin I converting enzyme inhibitory peptides from sake and sake lees. Biosci Biotechnol Biochem 1994;58:1767-71.
10. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 1971;20:1637-48.
11. Kim JH, Lee DH, Lee SH, Choi SY, Lee JS. Effect of *Ganoderma lucidum* on the quality and functionality of Korean traditional rice wine, *yakju*. J Biosci Bioeng 2004;97:24-8.
12. Seo DS, Kim JH, Ahn BH, Lee JS. Characterization of antidementia, cardiovascular and antioxidant functionalities in Korean traditional alcoholic beverages. Korean J Microbiol Biotechnol 2008;36:320-5.
13. Jeong SC, Kim JH, Kim NM, Lee JS. Production of antihypertensive angiotensin I-converting enzyme inhibitor from *Malassezia pachydermatis* G-14. Mycobiology 2005;33:142-6.
14. Athukorala Y, Jeon YJ. Screening for angiotensin I-converting enzyme inhibitory activity of *Ecklonia cava*. J Food Sci Nutr 2005;10:134-9.