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Quality Characteristics and Cardiovascular Activities of Korean Traditional Wines and Liquors

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Abstract The goal of this study was to screen and characterize the physiological functions of Korean traditional wines (TW) and liquors (TL). Forty-two TW and TL were collected and evaluated for quality and cardiovascular activities. Ethanol content ranged from 9.0%~41%, and pH ranged from 3.0~7.8, and they also contained 0.01% to 0.67% of total acid. Samples contained a maximum of 2.0% of crude protein and 0.1%~14.0% of reducing sugar. Commercial CM-wine showed the highest antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity, 85.9%. The greatest fibrinolytic activity and platelet aggregation inhibitory activity were also found in commercial CM-wine (31.8U) and commercial SS2-wine (38.6 %), respectively. Commercial SHBI-liquor showed the highest HMG-CoA reductase inhibitory activity, 78%. The ACE inhibitor from commercial CM-wine was a peptide compound and also showed an antihypertensive effect in spontaneous hypertensive rats at a dosage of 1.5 mg/kg.

Keywords: cardiovascular activities, angiotensin I-converting enzyme inhibitor, Korean traditional wines and liquors

Introduction

Korean traditional wines and liquors have long been made by classical methods using nuruk or koji (solid cultures of natural molds and koji mold producing amylase, respectively), cooked rice, yeast, and additives such as the roots or leaves of medicinal plants and herbs. Many research groups have studied ways to improve the quality of Korean traditional wines (rice and some medicinal plants-fermented alcoholic beverage) and liquors (medicinal fruit or plant-extracted alcoholic beverage). These studies have focused on various aspects such as changes in microbe and enzyme activity, the flavor and taste, the nutrients during the fermentation, the use of raw materials, standardization of the manufacturing process, storage, and marketing (1-2). Since the discovery of the functionality of Korean traditional wines in the form of chitooligosaccharides, which comes from koji mold, many traditional wines have been developed with a pharmaceutical or functional purpose. Simultaneously, the marketing of Korean traditional wines has gradually increased, reaching sales of 220 billion won (US$150 million). Korean traditional wines, however, have problems such as the lack of unique characteristics, lack of acceptability, and lack of physiological functionality. Few reports have been made on the cardiovascular activity of Korean traditional wines and liquors, though Saito and coworkers reported on the antihypertensive angiotensin I-converting enzyme inhibitor from sake lees (3-5). Therefore, it is necessary to develop new forms of Korean traditional wines with excellent acceptability, physiological functionality, and low alcohol toxicity.

The quality and functionality of Korean traditional wines have been reported (1) in wines made from dandelions (1), purple-fleshed sweet potatoes (6), chamomiles (7), acasias (8), ginseng (9), *Paecilomyces japonica* (10) and *Ganoderma lucidum* (11). The addition of medicinal plants or mushrooms into the mash increased the physiological functionality of Korean traditional wine. Among the many pharmacologically active, non-antibiotic natural products or traditional fermented food stuffs, those that show an antihypertensive, fibrinolytic, or platelet aggregation inhibitory effect, as well as cholesterol biosynthesis inhibitory effects, are particularly interesting. In this paper, we describe the quality and cardiovascular activities of selected Korean traditional wines and liquors.

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Materials and Methods

Materials The 42 types of Korean traditional wines and liquors used in this experiment were collected from wine stores in Daejeon or purchased directly from the manufacturer. Angiotensin I-converting enzyme (ACE) was extracted from rabbit lung acetone powder purchased from Sigma Chemical Co. (St. Louis, MO, USA), and its activity was determined with Hippuric-Histidine-Leucine (Hip-His-Leu) (Sigma) as a substrate. Spontaneously hypertensive rats (SHR), Sam:TacN(SHR)fBR, were purchased from Samtaco Bio-Korea Co.(Korea, O san city). Unless otherwise specified, all chemicals were of analytical grade.

Analysis of chemical properties The pH values were measured by a pH meter (Fisher Scientific, USA), and the titratable acidity was calculated as succinic acid (%) after titration with 0.1 N NaOH to pH 7.0. Ethanol was determined by an alcohol meter (Ceti Optical Instruments, Belgium) after steam distillation (10, 12). The crude protein and reducing sugar contents were determined according to the AOAC method (13).

Assay of cardiovascular activity After concentration of 50 ml of Korean traditional wine and liquor to 5 ml, its cardiovascular activity was determined as follows. First, the activity of ACE inhibition was assayed using Cushman and Cheung's method (14). A mixture containing a 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 3 units of ACE, and an appropriate amount of yakju, was preincubated for 10 min at 37°C. The reaction was initiated by adding 50 μl of Hip-His-Leu at a final concentration of 5 mM, and terminated after 30 min of incubation by adding 250 μl of 1.0 N HCl. The liberated hippuric acid was extracted with 1 ml of ethyl acetate, and 0.8 ml of the extract was dried using a Speed Vac Concentrator (EYELA Co., Japan). The residue was then dissolved in 1 ml of sodium borate buffer. The absorbance at 228 nm was measured to estimate the ACE inhibitory activity.

Fibrinolytic activity was assayed by the method of Fayek et al. (15). Each sample of 0.5 ml was added to 3 ml of substrate solution (0.6% fibrin in 0.1 M McIlvaine buffer, pH 7.0) and incubated at 40°C for 10 min. The reaction was stopped by adding 3 ml of 0.4 M TCA for 30 min and then filtered by Whatman filter paper No.2. The reaction mixture of 1 ml of the filtrates, 5 ml of 0.4 M Na_2CO_3, and 1 ml of 1 N Folin reagent was placed at room temperature for 30 min. The amount of tyrosine released from fibrin as substrate was determined from the tyrosine standard curve by measuring absorbance at 660 nm. One unit of activity was defined as the production of 1 μg of tyrosine per minute by 1 μl of the sample.

The platelet aggregation inhibitory activity was assayed using human platelets as follows (16). The platelet number was adjusted to 3×10^5 cells mm^{-3}. Platelet aggregation was measured turbidometrically with a spectrophotometer (Shimadzu, Japan) at 37°C. The platelet aggregation agents used in this experiment were PAF 1.5 μM and ADP 2.5 μM, and inhibitory activity of platelet aggregation was determined by adding extracts from Korean traditional wines and liquors.

The 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 (17). The reaction mixture in a volume of 0.5 ml 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 without HMG-CoA and extracts of Korean traditional wines and liquors. The assay was performed by adding HMG-CoA into one reaction mixture and adding HMG-CoA with each extract into the other reaction mixture 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 its oxidation in the absence of HMG-CoA from the rate observed with both substrates present.

HMG-CoA reductase inhibitory activity (%)

$$= 1 - \left(\frac{A_{340} \text{ of sample} - A_{340} \text{ sample of blank}}{A_{340} \text{ of control} - A_{340} \text{ sample of control}} \right) \times 100$$

Purification of ACE inhibitor and antihypertensive action in SHR Purification of the ACE inhibitor was performed by the methods of Lee *et al.* (18) using ultrafiltration, Sephdex-G-25 column chromatography, and HPLC. The antihypertensive action of the purified ACE inhibitor was also investigated according to previous methods (18). A dose of the purified ACE inhibitor from CM-wine, 1.5 mg/kg, was orally administered in spontaneously hypertensive rat. The systolic blood pressure of each rat was measured before administration and then afterwards at 15 min - 8 h from each rat tail, with a Blood Pressure Monitoring System (IWORX , USA).

Results and Discussion

Quality characteristics of commercial traditional wines (TW) and liquors(TL) Table 1 shows the chemical properties of TW and TL. TW and TL contained 9.0%~41.0% ethanol. Compared to fermented wines, most liquors contained more ethanol because a high proportion of ethanol, such as soju (ethanol content; 25%) and koryang-ju (ethanol content; 45%), is used in the preparation of Korean TL. The highest ethanol content was found in the SS-1 liquor, which was prepared with pine leaves and koryang-ju. The pH ranged from 4.0 to 5.0, except for BBJ-wine (pH 3.0) and DH-liquor (pH 7.8). TW and TL contained 0.01% to 0.67% of total acid. SG-wines showed the highest crude protein content of 2%, and HR-wine had the highest sugar content of 12.0%.

Cardiovascular activities of commercial TW and TL The angiotensin I-converting enzyme (ACE, dipeptidyl carboxypeptidase I, E.C. 3.4.15.1) regulates blood pressure by converting the inactive decapeptide, angiotensin I, to the potent vasoconstrictor octapeptide, angiotensin II, and inactivating the vasodilating nonapeptide, bradykinin, to raise blood pressure (19). Recently, various ACE inhibitors with antihypertensive effects have been isolated from the enzymatic digestion of food protein (20), sake and its by-products (5), cereals and legumes (21), and microbes such as yeast (22) and mushrooms (18).

Among commercial TW and TL, the antihypertensive ACE inhibitory activity was the highest in CM-wine at 85.9% (Table 2). CM-wine was brewed in a traditional way using glutinous rice and whole wheat. Furthermore, cereals and legumes (21), as well as sake lee (5), contain potent ACE inhibitors. Therefore, the high ACE inhibitory activity of CM-wine was probably caused by the production of ACE inhibitors from glutinous rice and wheat during fermentation (3, 4, 7). It also had higher activity than those of Korean traditional dandelion-wine (16.2%) (19), chamomile-wine (36.7%) (7), *Paecilomyces japonica*-wine (67.3%) (10), *Ganodenma lucidum*-wine (63.4%) (11), white wine (13.1%), and red wine (79.7%) (23).

The greatest platelet aggregation inhibitory activity was shown by SS-2-wine (38.6%), and the greatest fibrinolytic activity was shown by CM-wine (31.8 U). BI-liquor also had a high platelet aggregation inhibitory activity of 32.3 %. Although this fibrinolytic activity was higher than in Korean traditional purple-fleshed sweet potato-wine (20 U), dandelion-wine (18.7 U) (19), *Paecilamyces japonica*-wine (11.2 U) (10), and chamomile-wine (9.0 U) (7), it is

Table 1. Chemical properties of Korean traditional wines and liquors

Traditional wines and liquors	Type[a]	Ethanol content (%)[b]	pH	Total acid (%)[c]	Volatile acid(%)	Crude protein (%)	Reducing sugar (mg/ml)
DCHC[d]	TW	12	3.69	0.314	0.0102	0.6	10.0
YJ	TW	16	4.11	0.441	0.0282	1.6	6.5
GH	TW	14	3.78	0.672	0.0370	2.0	1.3
HR	TW	13	3.71	0.257	0.0018	0.6	6.4
GH	TW	14	4.11	0.278	0.0072	0.9	1.7
PS	TW	15	4.22	0.261	0.0084	1.3	10.8
SJDB	TW	13	4.51	0.214	0.0024	1.2	8.0
MR	TW	12	3.73	0.271	0.0072	0.1	11.0
BS	TW	13	3.49	0.325	0.0030	0.3	10.7
SSC	TW	13	3.27	0.330	0.0036	0.4	7.6
SG	TW	16	4.77	0.254	0.0114	2.0	8.0
DC-1	TW	14	4.21	0.326	0.0108	1.8	9.0
GG-1	TW	15	4.51	0.157	0.0069	1.0	12.0
IS	TW	11	3.91	0.319	0.0072	1.1	10.5
GG-2	TW	9	3.82	0.366	0.0402	0.7	8.5
GG-3	TW	13	3.45	0.330	0.0096	0.7	4.0
CM	TW	16	5.03	0.224	0.318	1.1	11.0
BG	TW	16	3.63	0.556	0.9844	1.3	9.0
BBJ	TW	21	3.01	0.761	0.9662	n.d	10.5
SJOG	TW	17	4.72	0.208	0.9648	1.0	13.0
SL	TW	11	4.07	0.401	0.9767	1.7	10.0
SS-2	TW	12	4.58	0.148	0.9769	1.2	12.0
GJ	TW	13	3.40	0.283	0.9832	0.3	12.5
YWC	TL	25	5.90	0.012	0.0012	n.d[e]	5.4
HH	TL	18	3.58	0.092	0.0027	n.d	10.7
SSY	TL	17	6.02	0.001	0.0012	n.d	7.0
DD	TL	22	3.95	0.104	0.0033	0.6	8.0
SS-1	TL	41	4.48	0.012	0.0036	n.d	1.6
PP	TL	26	6.01	0.001	n.d	n.d	1.6
DH[d]	TL	23	7.76	n.d	n.d	n.d	0.1
BHS	TL	39	4.43	0.096	0.0147	n.d	7.0
BR	TL	33	3.52	0.253	0.0627	n.d	8.4
W	TL	24	3.64	0.049	0.0026	n.d	9.2
BI	TL	38	3.79	0.074	0.0288	n.d	8.2
DC-2	TL	20	5.53	0.018	0.0024	n.d	12.7
YG	TL	25	3.87	0.043	0.9688	n.d	12.5
SHBI	TL	35	4.51	0.018	0.9358	n.d	13.0
H	TL	31	3.89	0.061	0.9511	n.d	0.8
YM	TL	26	4.98	0.041	0.9732	0.1	14.0
CS	TL	22	4.16	0.083	0.9609	n.d	8.0
JYC	TL	42	4.07	0.024	0.9159	n.d	13.0
SS-3	TL	26	4.74	0.011	0.9611	n.d	10.8

[a]TW : traditional wine, TL : traditional liquor
[b]% : v/v % except crude protein (w/v %)
[c]Total acid described as succinic acid
[d]Initial names of commercial traditional wines or liquors
[e]n.d : not determined

probably less effective for preventing or curing thrombosis because of its low activity.

HMG-CoA reductase inhibitory activity, which inhibits cholesterol biosynthesis, was high in SHBI-liquor (78.0%), PP-liquor (67.9%), and SS-3-liquor (66.0%). These high levels of HMG-CoA reductase inhibitory activity may be caused by the extraction of ethanol-soluble cholesterol synthesis inhibitors from the pine leaves and mulberry leaves used in the preparation of SHBI, PP, and SS-3-liquors. The results indicate that Korean traditional wines and liquors have highly valuable cardiovascular activity, such as antihypertensive, antithrombosis and antihypercholesterolemia agents, probably due to the extraction of various substrates from medicinal plants and herbs during fermentation (1).

Purification of ACE inhibitor from commercial CM-Korean traditional wine The ACE inhibitor from CM-wine, a representative cardiovascular agent in TW and TL, was purified by ultrafiltration, Sephadex G-25 column

Table 2. Cardiovascular activities of Korean traditional wines and liquors

Traditional wines and liquors	ACE inhibitory activity (%)[a]	Fibrinolytic activity (U)	Platelet aggregation inhibitory activity (%)	HMG-CoA reductase inhibitory activity (%)	Glutathione S-transferase activity (%)
DCHC	51.0	n.d[b]	n.d	12.4	-[c]
YJ	73.0	n.d	n.d	n.d	-
GH	67.0	9.6	15.3	8	-
HR	41.8	2.0	26.6	16	-
GH	75.3	9.8	n.d	42	-
PS	71.6	n.d	n.d	n.d	-
SJDB	77.2	n.d	n.d	12.4	165.7
MR	n.d	n.d	n.d	50	-
BS	49.1	7.2	n.d	n.d	-
SSC	32.0	n.d	n.d	25	-
SG	59.2	26.8	n.d	30	-
DC-1	64.4	12.6	n.d	n.d	-
GG-1	69.1	n.d	n.d	n.d	-
IS	70.0	n.d	n.d	n.d	-
GG-2	70.0	n.d	n.d	51	-
GG-3	69.0	n.d	15.3	n.d	-
CM	85.9	31.8	19.8	56	-
BG	59.6	n.d	n.d	n.d	-
BBJ	19.2	n.d	n.d	29	-
SJOG	73.5	12.4	11.3	32	-
SL	66.8	n.d	n.d	n.d	-
SS-2	65.8	13.2	38.6	47	-
GJ	44.1	n.d	19.0	15	-
YWC	n.d	n.d	n.d	35	-
HH	2.3	n.d	26.3	10	-
SSY	15.8	n.d	n.d	n.d	107.2
DD	13.5	n.d	n.d	2	-
SS-1	55.3	n.d	n.d	8.5	-
PP	n.d	n.d	n.d	67	-
DH	n.d	n.d	n.d	26	-
BHS	n.d	n.d	n.d	36	-
BR	35.2	2.8	n.d	n.d	-
W	4.3	n.d	n.d	32	-
BI	n.d	n.d	32.3	10	-
DC-2	n.d	n.d	4.8	20	-
YG	24.3	1.2	n.d	21	-
SHBI	39.7	3.0	n.d	78	-
H	2.0	n.d	n.d	8	-
YM	4.0	n.d	n.d	11	-
CS	29.0	2.8	n.d	48	-
JYC	n.d	n.d	n.d	35	-
SS-3	n.d	n.d	14.0	66	-

[a] %: w/w%
[b] n.d : not detected
[c] - : below 100%(control)

chromatography, and HPLC (18). Table 3 summarizes the purification of ACE inhibitors from CM-Wine. The ACE inhibitory activity of the filtrates from the 5,000 M.W. cut-off ultrafiltration of commercial CM-Wine was 0.31 mg of IC_{50}. After Sephadex G-25 column chromatography, the active fraction showed 0.19 mg of IC_{50} (GF1). The active fraction was collected and subjected to preparation by reverse phase HPLC using μ Bondapack C18 column. One peak containing ACE inhibitory activity was obtained (IC_{50}; 0.095 mg). After that, two reverse-phase HPLC were performed for the active fraction. When subjected to reverse-phase HPLC using a V protein/peptide reversed-

Table 3. Summary for the purification of ACE inhibitor from CM-wine

Purification steps	ACE inhibitory activity (IC_{50}; mg)	Solid yield (%)
Water extract	0.31	100
Ultrafiltration	0.28	72.1
Sephadex G-25	0.24	34.9
Prep. RP-HPLC	0.16	3.9
First RP-HPLC	0.05	2.3
Second RP-HPLC	0.04	0.3

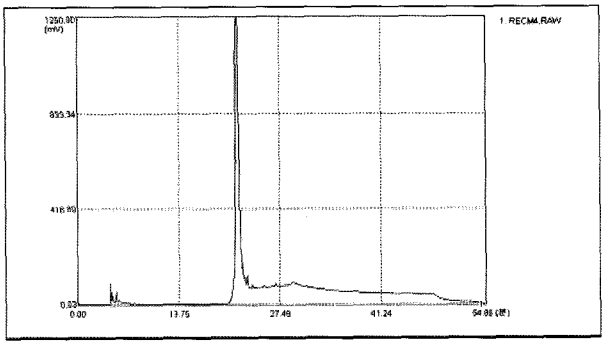

Fig. 1. Reverse phase HPLC of GF1 from sephadex G-25 column chromatography.

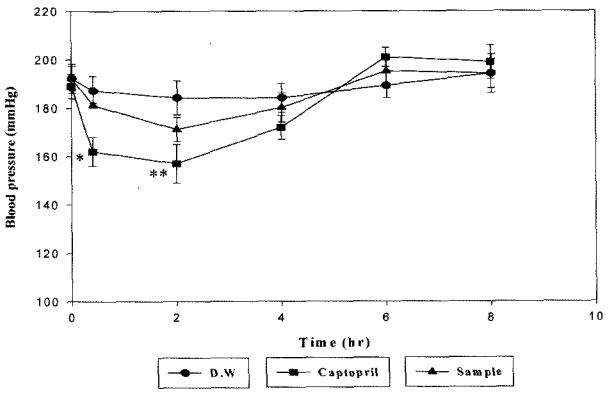

Fig. 2. Effect of orally administered ACE inhibitor from CM-Wine on blood pressure in SHR. ▲, ACE inhibitor 1 mg/kg, ■, positive control (captopril) 1.5 mg/kg, ● , negative control (DW). *,** significantly different from test group at $p<0.05$ by Tukey's test.

phase 218TP column, a single peak was eluted (Figure 1). After the purification step, an ACE inhibitor with an IC_{50} of 0.04 mg was obtained, and the yield was 0.3%. It was a peptide compound, showing a maximum absorption spectra of 215 nm.

Antihypertensive action of purified ACE inhibitor As shown in Fig. 2, the average blood pressure of the ACE inhibitor group was 195 mmHg just before administration. At 2 h after ACE inhibitor administration (1.5 mg/kg), blood pressure decreased to 178 mmHg, and later returned to the average blood pressure. These results were similar to the commercial antihypertensive drug, captopril (195 mm → Hg160 mmHg), suggesting that the purified ACE inhibitor produces a antihypertensive effect in SHR at a dosage of 1.5 mg/kg.

Conclusion

Korean traditional wines and liquors are brewed using rice, nuruk, or koji with additives such as roots or leaves of oriental medicinal plants and herbs. They contain various nutrients and several medicinal compounds for preventing disease. However, the nutrient and physiological functions need to be investigated in more detail. Korean traditional wines and liquors contained a maximum of 2.0% of crude protein and 0.1% to 14.0% of reducing sugars.

Antihypertensive ACE inhibitory activities were high in most Koran traditional wines and liquors. In particular, 85.9% of ACE inhibitory activity was found in CM-wine. Fibrinolytic and platelet aggregation activity of TW and TL were low, whereas HMG-CoA reductase inhibitory activity, which is related to antihypercho-lesterolemia, showed the highest (78%) in SHBI-liquor. The ACE inhibitor of CM-wine was purified and characterized as a peptide compound with antihypertensive activity in a spontaneous hypertensive rat test. We concluded that Korean traditional wines and liquors are high-value alcoholic beverages with various nutrients and beneficial cardiovascular agents, such as antihypercholesterolemia substances. This study provides data for understanding the structure-function relationship of ACE inhibitors in Korean traditional wine.

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