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Identification of Character-impact Aroma Compounds and Comparisons of Sensory Attributes of Traditional Korean Medicinal Rice Wines Brewed with Functional Herbal Powders or Extracts

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Abstract

Rice wine was prepared with medicinal plants or plant extracts to obtain a value added nutritious alcoholic tonics. Powders of ten medicinal plants (PTM) or aqueous extracts prepared from them (ATM) were added during the initial stage of fermentation. Aroma compounds of rice wine (control) and wines containing PTM or ATM were isolated by liquid-liquid continuous solvent extraction (LLCSE) and analyzed by gas chromatography-olfactometry and aroma extract dilution analysis (AEDA). Desirable aroma compounds: acetaldehyde (sweet, ethereal), benzaldehyde (sweet, fragrant), ethyl acetate (sweet) and ethyl octanoate (sweet, ethanolic) had the highest $\log_3$-flavor dilution (FD) factors in ATM. Results of sensory evaluation demonstrated that intensities of undesirable aroma attributes, such as koji and yeasty notes in control, and raw medicinal herb notes in PTM, were lowest in wine with ATM. Wines made with ATM had the most attractive aroma attributes among the three different traditional Korean medicinal wines.

Key words: medicinal wines, flavor, sensory evaluation, aroma extract dilution analysis

INTRODUCTION

Medicinal plants have a long history of use throughout Asia as traditional medical treatments and tonics without serious side effects. Traditionally, the leaves, roots, fruits or flowers of medicinal plants have been extracted by using boiling water for two or three hours with low heat. The water extracts are concentrated and consumed as such or made into medicinal pills. There are ten especially popular medicinal plants used in the preparation of tonics: *Glycyrrhiza uralensis*, *Angelica gigas*, *Pachyma hoelen*, *Atractylodes japonica*, *Paeonia japonica*, *Rehmannia glutinosa*, *Cnidium officinale*, *Panax ginseng* C. A. Meyer, *Cinnamomum loureirii* (cinnamon), and *Astragalus membranaceus*. The root of *G. uralensis* (licorice) is one of the most frequently used plants for traditional natural medicines in Asia. Hatano (1) isolated the phenolic compounds including flavonoid glycosides from licorice. Ahn et al. (2) isolated immunostimulating components from the root of *A. gigas*, and *P. japonica* has been prescribed for the treatment of various womens diseases in Asia (3). Kim et al. (4) demonstrated that an aqueous extract of *R. glutinosa* inhibited a systematic allergic reaction. An acidic polysaccharide isolated from the rhizome of *C. officinale* was shown to have anti-complement activities (5). *P. ginseng* C. A. Meyer, also known as ginseng or insam, is known to have anti-stress, anti-fatigue, hypoglycemic and antioxidant properties (6). *A. japonica* has been used in traditional oriental medicine for the treatment of water retention (7), and Konno et al. (8) isolated hypoglycemic glycans from its rhizomes. Some of these medical plants have been shown to reduce alcohol absorption from the gastrointestinal system (9).

Rice wines, having unique flavors and aromas, have been consumed as high quality nutritious alcoholic beverages throughout Asia. We postulated that brewing rice wine with medicinal plants or plant extracts can confer functional properties to the wine with benefits including reducing hangover symptoms, such as headache, and hepatic insufficiency; without impairing flavor. In this study, the powders and aqueous extracts of ten medicinal plants were used in making rice wines intended to be highly acceptable in terms of flavor and aroma. The character-impact aroma compounds and sensory attributes of the wines were compared to determine which herbs or herbal extracts can be used to contribute functional benefit and simultaneously contribute desirable flavor and aroma.

MATERIALS AND METHODS

Materials

Rice wine was made with "Chu-Chung" general rice, *As-*

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pergillus kawachi, Aspergillus shirousami (Chung-Nam National University, Korea) and *Saccharomyces cerevisie* (Midochemical Co. Korea) were used for fermentation. Ten medicinal plants: *G. uralensis, A. gigas, P. hoelen, A. japonica, P. japonica, R. glutinosa, C. officinale, P. ginseng* C. A. Meyer, *C. loureirii*, and *A. membranaceus*, were purchased from the marketplace at Gum-San (Korea). All of the plants, except *R. glutinos*, which remained in whole form, were ground and passed through a 200 mesh sieve. Aroma standards were purchased from Sigma (St. Louis, MO, USA).

Preparation of rice koji

Two hundred grams of rice were soaked for 24 hours in tap water and steamed at 100°C for 20 min. The steamed rice was cooled to 30°C, then three grams of microbial seed, which consisted of *Asp. kawachii* and *Asp. Shirousami* in wheat bran, were inoculated into the mixture and incubated for two days at 30°C (10).

Fermentation

During the first fermentation stage, two hundred grams of rice koji were mixed with 2 g of the yeast, *Sacch. Cerevisiae*, and 400 mL of tap water and then fermented for 2 days at 30°C (10). During the second stage, the fermented rice koji, obtained from the first stage, was mixed with 800 g of steamed rice and added to either plain water (control), powder one of the medicinal plants (PTM), or the aqueous extract (ATM) of one of the ten medicinal plants. Twenty-five milligrams of each of the nine medicinal plants was added directly for making PTM. Aqueous extracts were prepared by adding 25 mg of each medicinal plant to 1000 mL of distilled water and mixing and refluxing for 2 hours at 100°C. The aqueous extracts were then filtered and made up to 1225 mL with sterilized distilled water. The composition of the medicinal rice wines are shown in Table 1. The mixtures were brewed for 60 days at 18°C. After brewing, the wines were centrifuged at 3000 rpm for 20 min. and stored at -20°C in glass bottles sealed with Teflon-lined screw caps. Stored samples were thawed overnight in a refrigerator and volatiles isolated by LLCSE.

Isolation of character-impact aroma by using liquid-liquid continuous solvent extraction (LLCSE)

A mixture of 300 mL brewed rice wine, 818 μg of 3-heptanol, and 200 mL of deodorized water were continuously extracted with 250 mL methylene chloride for 6 hours at room temperature. Deodorized water was prepared by boiling distilled water in an open flask until the volume was decreased one-third. After extraction, the solvent layer was collected and kept overnight at -20°C to remove bulk water as crystallized ice. The extract was then passed through a column containing 10 g of anhydrous sodium sulfate and the volume further reduced to 2 mL under a stream of nitrogen, and then further concentrated by passing through 1 g of anhydrous sodium sulfate under a stream of nitrogen reducing the volume to 100 μL. The dried extract was analyzed by GC-MS analysis and then serially diluted (1:3) in methylene chloride for aroma extraction dilution analysis by GC-O.

Aroma extraction dilution analysis (AEDA) of LLCSE extracts by gas chromatography-olfactometry (GC-O)

AEDA was performed as described by Grosch (11) and Cadwallader et al. (12). The serially diluted extracts (1:3) were injected (1 μL, splitless) into a GC-O system consisting of a M600D GC (Young-In, Korea) equipped with a flame ionization detector (FID) and an olfactometer (sniffing port). Separations were performed on a DB-5MS column (60 m length×0.25 mm i.d.×0.25 m film thickness ; J & W Scientific, USA) and a HP-WAX (30 m length ×0.25 mm i.d.×0.20m film thickness, Hewlett Packard, USA) capillary column. Oven temperature for the DB-5MS column was programmed to increase from 35°C to 220°C at a rate of 6°C/min with initial and final hold times of 3 and 20 min, respectively. Oven temperature for the HP-WAX column was programmed to increase from 35°C to 200°C at a rate of 5°C/min with initial and final hold times of 3 and 20 min, respectively. Injector temperature was 200°C. The olfactometer transfer lines and FID detection

Table 1. Composition of additives for making traditional Korean medicinal rice wines

Brewing stage	Mixed material	Control[1)]	ATM[2)]	PTM[3)]
First stage	Rice koji (g)	200	200	200
	Yeast (g)	2	2	2
	Water (mL)	400	400	400
Second stage	Rice (g)	800	800	800
	Water (mL)	1225	-	1200
	Medicinal plant powders (g)	-	-	25
	Medicinal plant extracts (mL)	-	1225	-

[1)]Traditional Korean rice wine control.
[2)]Wine made with aqueous extract of ten medicinal plants.
[3)]Wine made with powders of ten medicinal plants.

emperatures were held at 250°C. The olfactometer was upplied with humidified air at 30 mL/min. GC-O was performed by three trained panelists who were instructed to ssign odor properties to each compound detected in each xact dilution. A flavor dilution (FD) factor for each odorant was assigned based on the highest dilution at which t was detected by GC-O. The FD-factors of the odorants re presented as the average log_3 FD-factors of six determiations.

Gas chromatography and mass spectrometry (GC-MS) analysis

Two ml of LLCSE extracts was injected (splitless mode, 30s valve-delay) into the fused silica DB-5MS and HP-WAX capillary columns and analyzed by a Saturn GC-MS ystem (Varian, USA). Helium was used as the carrier gas vith a constant flow rate of 1.0 mL/min. GC oven temperature for the DB-5MS column was programmed to increase rom 35°C to 220°C at a rate of 2°C/min with an initial old time of 5 min and final hold time of 30 min. GC oven emperature for HP-WAX was programmed to increase rom 35°C to 200°C at rate of 2°C/min with initial hold ime of 5 min and final hold time of 30 min. Injector temerature was 200°C. MSD capillary interface temperature vas 170°C, ionization energy was 70 eV, mass range was 3~450 a.m.u., and scan rate was 3 scans/s. Duplicate injections were performed. Peaks were tentatively identified y computer matching (using Wiley library search); and hen confirmed by matching of retention indices (13), mass pectra, and odor characteristics with reference standards.

Sensory evaluation

Sensory evaluation was performed by quantitative descriptive analysis (QDA) (14). Eight panelists were selected from among graduate students of Chungnam National University and trained for five sessions. Descriptive terms were discussed and selected by panelists. Ratings were scored on a line scale from 0 (weak) to 15 (strong). Ratings rom the eight panelists were pooled and presented as mean alues. Statistical analysis was performed using a SAS program (SAS Institute Inc., Release 6.12, Cary, NC, USA). Significant differences between samples were compared y least significant difference values.

RESULTS AND DISCUSSION

Instrumental analysis

Thirty-two aroma compounds were detected by GC-O n the HP-WAX capillary column, 24 of which were positively identified by matching odor, mass spectra and retention indices with reference standards (Table 2). The other compounds in the table were considered unknown. Acetaldehyde (sweet), ethyl acetate (sweet), ethanol (ethanolic), 2,3-butandione (rotten, buttery), 2,3-pentandione (rotten, buttery), 2-methylpyrazine (nutty), 3-heptanone (rotten, citrus), heptanal (mushroomy, planty), 3-hydroxy-2-butanone (rotten citrus), 2-acetyl pyrazine (popcorn), hexanol (citrus), ethyl octanoate (ethanolic, sweet), benzaldhyde (sweet, fragrance), ethyl-hydroxycarproate (ashy, ethereal, citrus), methyl caprate (citrus) and phenethyl alcohol (sweet, floral) were all detected in control, ATM and PTM wines with comparable high Log_3-FD factors. Twenty chemical compounds were detected by GC-O on the DB-5MS capillary column and are presented in italics in Table 2. The compounds acetaldehyde, ethyl acetate, ethanol, 2,3-butanedione, 2,3-pentanedione, carpronaldehyde, 2-methylpyrazine, 3-heptanone, 3-hydroxy-2-butanone, benzaldehyde, and phenethyl alcohol were also detected in control, ATM and PTM wines with high log_3-FD factors. Acetaldehyde, ethyl acetate, ethanol, ethyl octanoate, benzaldehyde, ethyl hydroxycarproate, methylcaprate and phenethyl alcohol are possibly desirable aroma components of traditional Korean medicinal rice wines (TKMRW). Meanwhile, other compounds such as 2,3-butandione, 2,3-pentandione, 2-methylpyrazine, 3-heptanone, heptanal, 3-hydroxy-2-butanone, 2-acetyl pyrazine and hexanol might have an undesirable effect. Ethyl acetate, ethyl octanoate, benzaldehyde, ethyl hydroxycaproate and phenethyl acetate, which impart desirable fruity ester aromas to TKMRW, had the highest Log_3-FD factors in the ATM wines. The presence of these esters is in agreement with a report by Maarse (15), who mentioned that esters were induced by enzymatic fermentation in the presence of organic acids, which suggests that the esters may be produced by the fermentation of organic acids.

The (possibly undersireable) aroma compounds; 2,3-butandione, 3-heptanone, 3-hydroxy-2-butanone, 2-methyl-3-furanthiol, 3-(methylthio) propanal and 1-octen-3-ol, were present in the control wine. 2,3-butandione is well known as an aroma compound that develops during yeast fermentation (16). The Log_3-FD factors of these aroma compounds were reduced in ATM and PTM wines, which may be explained by the medicinal herb aroma masking and/or binding these aroma compounds.

Some unknowns, such as no. 7 (herb, smoky), no. 10 (thujone), no. 11 (raw potato), no 19 (herb), no. 20 (rhmania glutonosa), no. 21 (medicinal herb) and no. 24 (garlic, planty), no.13 (3-methyl-1-butanol; sweet) and no.18 ([E]-2-heptenal; planty, almond, seedy) were detected in ATM and PTM wines. The Log_3-FD factors of these aroma compounds were higher in PTM wines than in ATM wines. Although some unknown aroma compounds had comparably high Log_3-FD factors, these could not be identified in this study.

Other characteristic aroma compounds only detected

Table 2. Comparison of average log_3-flavor dilution factors of character-impact aroma compounds in traditional Korean medicinal rice wines

No	RI[1]	Odor description	Chemical compound	Identification methods	Control[6]	ATM[7]	PTM[8]
1	*1.3[2]	Sweet	Acetaldehyde	MS, Odor, RT[5]	5.0[9](0.0)[10]	3.3(2.1)	4.0(1.7)
	*(3.2)[3]				***2.3***[11]***(0.7)***[12]	***4.5(0.7)***	***4.0(1.4)***
2	885	Sweet	Ethylacetate	MS, Odor, RI	4.7(0.6)	4.7(0.6)	5.0(0.0)
	*(7.5)				***3.3(0.0)***	***5.0(0.0)***	***3.5(2.1)***
3	947	Ethanolic	Ethanol	MS, Odor, RI	5.0(0.0)	5.0(0.0)	5.0(0.0)
	*(5.3)				***4.7(2.1)***	***4.5(0.7)***	***4.0(0.0)***
4	960	Rotten buttery	2,3-Butandione	MS, Odor, RI	5.0(0.0)	3.7(2.3)	2.7(0.6)
	*(6.7)				***4.0(0.0)***	***3.3(0.0)***	***3.3(1.5)***
5	1009	Pungent ethereal	Ethylbutyrate	MS, Odor, RI	4.7(0.6)	-	-
	(796)				***1.7(0.7)***	-	-
6	1021	Rotten, buttery	2,3-Pentandione	MS, Odor, RI	5.0(0.0)	4.0(1.7)	3.7(1.5)
	(727)				***4.0(1.0)***	***4.0(0.0)***	***4.0(1.4)***
7	1025	Herb, smoky	Unknown	Odor	-	1.0(0.0)	3.7(1.5)
	-[4]				-	-	-
8	1057	Fresh planty	Carpronaldehyde	MS, Odor, RI	-	1.0(0.7)	-
	(812)				***4.3(1.2)***	***4.0(1.4)***	***5.0(0.0)***
9	1068	Nutty	2-methylpyrazine	MS, Odor, RI	3.0(2.6)	4.0(1.0)	3.7(2.3)
	(837)				***4.0(1.0)***	***4.0(1.4)***	***3.0(2.8)***
10	1076	Thujone	Unknown	Odor	-	3.0(1.0)	4.0(1.4)
	-				-	-	-
11	1100	Raw potato	Unknown	Odor	-	4.0(1.7)	3.0(2.0)
	-				-	-	-
12	1121	Rooten, citrus	3-Heptanone	MS, Odor, RI	4.0(1.7)	3.0(2.8)	4.7(0.6)
	(858)				***3.3(2.1)***	***4.0(1.4)***	***3.7(2.3)***
13	1142	Sweet	3-Methyl-1-butanol	MS, Odor, RI	-	4.3(1.2)	3.3(2.0)
	-				-	-	-
14	1185	Mushroomy, planty	Heptanal	MS, Odor, RI	4.7(0.6)	4.3(1.2)	2.3(1.5)
	-				-	-	-
15	1260	Rotten citrus	3-Hydroxy-2-butanone	MS, Odor, RI	5.0(0.0)	4.3(1.2)	4.0(1.0)
	(748)				***4.0(1.4)***	***3.7(1.2)***	***3.5(0.7)***
16	1274	Popcorn	2-Acetylpyrazine	Odor, RI	2.0(1.0)	4.3(0.6)	3.0(1.0)
	-				-	***4.5(0.7)***	-
17	1287	Mushroomy	1-Octen-3-one	MS, Odor, RI	1.7(0.6)	-	-
	-				-	-	-
18	1311	Planty, almond, seedy	[E]-2-Heptenal	MS, Odor, RI	-	2.5(2.1)	4.3(1.2)
	-				-	-	-
19	1324	Herb	Unknown	Odor	-	4.3(1.2)	4.3(1.2)
	-				-	-	-
20	1352	Rhmania glutinosa	Unknown	Odor	-	2.3(1.5)	4.7(0.6)
	-				-	-	-
21	1379	Medicinal herb	Unknown	Odor	-	3.7(1.2)	4.0(1.7)
	-				-	-	-
22	1393	Citrus	Hexanol	MS, Odor, RI	2.3(2.3)	2.0(1.0)	3.3(1.5)
	-				-	-	-
23	1411	Ethanolic, sweet	Ethyl octanoate	MS, Odor, RI	2.0(1.4)	4.0(1.7)	3.0(2.0)
	-				-	-	-
24	1440	Garlic, planty	Unknown	Odor	-	4.3(0.6)	5.0(0.0)
	-				-	-	-
25	1457	Citrus, ethereal, sour	Acetic acid	MS, Odor, RI	1.5(0.7)	5.0(0.0)	-
	-				-	-	-
26	1465	Sweet, candy	Unknown	Odor	4.0(1.4)	4.0(1.7)	-
	-				-	-	-
27	1485	Sweet, fragrant	Benzaldehyde	MS, Odor, RI	3.5(0.7)	3.7(1.5)	3.3(2.1)
	(953)				***2.7(2.1)***	***4.5(0.7)***	***3.3(2.0)***
28	1544	Ashy, ethereal, citrus	Ethyl hydroxycaproate	MS, Odor, RI	4.0(1.4)	5.0(0.0)	3.0(1.7)
	-				-	-	-
29	1580	Citrus	Methyl caproate	MS, Odor, RI	2.7(1.5)	3.3(2.1)	3.0(1.7)
	(1338)				-	***3.5(2.1)***	***3.3(0.6)***
30	1641	Nutty, faint	Diethyl succinate	MS, Odor, RI	4.3(1.2)	-	2.7(1.5)
	-				-	-	-

Table 2. Continued

No	RI[1]	Odor description	Chemical compound	Identification methods	Control[6]	ATM[7]	PTM[8]
31	1829	Citrus, sweet	2-Methyl-2-butenoic acid	MS, Odor, RI	-	3.3(1.5)	-
	-				-	-	-
32	1890	Sweet, floral	Phenethyl alcohol	MS, Odor, RI	2.5(2.1)	3.0(2.0)	4.0(1.0)
	(1139)				*2.0(1.8)*	*3.0(2.8)*	*3.0(1.0)*
33	-	Cooked nutty, meaty	2-Methyl-3-furanthiol	Odor, RI	-	-	-
	(891)				*4.5(0.7)*	*2.3(0.7)*	*2.3(0.6)*
34		Seedy, cooked potato	3-(Methylthio)-propanal	Odor, RI	-	-	-
	(905)				*4.0(1.0)*	*3.5(0.7)*	*3.0(2.8)*
35		Mushroomy	1-Octen-3-ol	MS, Odor, RI			
	(974)				*4.5(0.7)*	*3.0(0.7)*	*3.3(2.1)*
36		Raw medicine,	6-Methyl-5-hepten-2-one	MS, Odor, RI	-	-	-
	(987)	herbaceous			-	-	*5.0(0.0)*
37		Planty, beany	3-Octanol	MS, Odor, RI	-	-	-
	(996)				*3.3(0.0)*	*4.5(0.7)*	*3.7(1.5)*
38		Sweet, fruity	Phenethyl acetate	MS, Odor, RI	-	-	-
	(1317)				*2.3(0.7)*	*3.5(2.1)*	*3.0(1.4)*

f) Values in italics are related to GC-O on DB-5MS capillary column. *Retention time.
[1]Retention indices (RI) calculated from GC-MS data and calculated by the method of van den Dool and Kratz (13).
[2]RI on HP-WAX capillary column.
[3]RI on DB-5MS capillary column.
[4]Not detected.
[5]Compounds identified by matching of retention indices of standards on GC-O and mass spectrum on GC-MS.
[6]Traditional Korean medicinal rice wine.
[7]Wine made with aqueous extracts of ten medicinal plants.
[8]Wine made with powders of ten medicinal plants.
[9]Average $\log_3$-Flavor dilution factor of three panelists on HP-WAX capillary column.
[10]Standard deviation.
[11]Average $\log_3$-Flavor dilution factor of three panelists on DB-5MS capillary column.
[12]Standard deviation.

vhen using the DB-5MS capillary column included: 2-nethyl-3-furanthiol (cooked nutty, meaty), 3-(methylthio) ropanal (seedy, cooked potato), 1-octen-3-ol (mushroomy), -methyl-5-hepten-2-one (raw medicine, herbaceous), 3-ctanol (planty, beany) and phenethyl acetate (sweet, flo-al). All of these aroma compounds, except phenethyl ace-ate, might have an undesirable effect in TKMRW. Ethyl utyrate (pungent ethereal) and 1-octen-3-one (mushroomy) vere only detected in the control wine.

The instrumental analysis results indicated that the ad-ition of medicinal herbs reduces aroma compounds pro-uced during fermentation, and that aqueous extracts of nedicinal plants yield wines with less of a medicinal aro-na than wines made with powdered whole herbs.

Sensory evaluation

Sensory panelists chose seven terms to describe TKMRW romas: steamed medicinal herb, koji, sour, yeast, sweet lcohol, caramel and malt extracts, and raw medicine herb. he aroma profiles, based on the consensus of the panels, re depicted as spider web plots in Fig. 1. Average ratings nd significance among treatments are shown in Table 3. he steamed medicine herb aroma term was scored highest n PTM wine, followed by ATM wine and control, which ould explain why using aqueous extracts in making

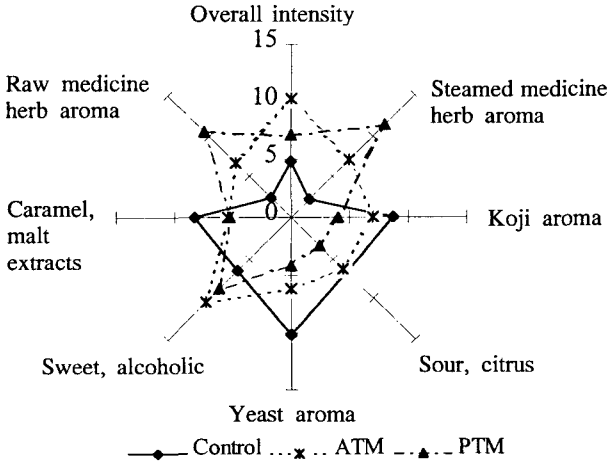

Fig. 1. Aroma sensory profiles among the traditional Korean medicinal rice wines. Control: Traditional Korean medicinal rice wine. ATM: Wine made with extracts of ten medicinal plants. PTM: Wine made with powders of ten medicinal plants.

TKMRW might reduce the herb aroma notes.

The average intensities of koji, sour, and citrus aromas were lowest in PTM wine, and were not significantly different between control and ATM. Yeast and malt extract aromas were highest in control, and did not differ between ATM and PTM wines. The raw and steamed medicinal herb aromas might reduce koji, yeast and malt extract aromas

Table 3. Sensory comparison of aroma attributes among the traditional Korean medicinal rice wines

Aroma description	Control[1)]	ATM[2)]	PTM[3)]
Steamed medicine herb aroma	2.3[c4)]	7.1[b]	11.2[a]
Koji aroma	8.7[a]	7.0[a]	4.0[b]
Sour, citrus	6.7[a]	6.3[a]	3.4[b]
Yeast aroma	10.1[a]	6.1[b]	4.1[b]
Sweet, alcoholic	6.4[b]	10.2[a]	8.8[ab]
Caramel, malt extracts	8.3[a]	5.4[b]	5.2[b]
Raw medicine herb aroma	2.4[c]	6.6[b]	10.4[a]
Overall intensity	4.9[b]	10.2[a]	7.1[b]

[1)]Traditional Korean medicinal rice wine.
[2)]Wine made with extracts of ten medicinal plants.
[3)]Wine made with powders of ten medicinal plants.
[4)]Statistical results. Numbers in rows having different superscripts are significantly different at $p \leq 0.05$.

by either binding the aroma compounds or producing a masking effect.

Sensory evaluation demonstrated that the addition of ATM reduced undesirable koji and yeast aromas as well as the steamed medicinal herb aroma of the wines with added herbal powders. Both instrumental and sensory analyses provide the evidence that traditional Korean medicinal rice wines can be fermented into functional herbal tonics with the addition of aqueous herbal extracts that impart both functional properties and desirable organoleptic qualities.

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