Acetaldehyde contents and quality characteristics of commercial alcoholic beverages

Kwang-Seup Shin¹ · Jeung-Hee Lee¹

Received: 10 August 2018 / Revised: 8 January 2019 / Accepted: 16 January 2019 / Published online: 6 February 2019
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Abstract The quality characteristics of commercial alcoholic beverages (yakju, diluted soju, and fruit wines) were evaluated by assessing pH, total acidity, acetaldehyde, SO₂, and flavonoid content. pH level was the highest in diluted soju, and total acidity and flavonoid content were higher in fruit wines compared to others. SO₂ was detected only in fruit wines, and the total SO₂ content was higher in the order of white wine > red wine > plum wine. Acetaldehyde contents were different according to the type of alcoholic beverage and analytic method of titration, enzymatic, headspace-GC and OIV methods. In fruit wines, acetaldehyde contents were significantly different according to analytic method ($p < 0.05$), and acetaldehyde bound to SO₂ affected its quantification, resulting in lower acetaldehyde contents assessed by titration and headspace-GC than enzymatic and OIV methods. Therefore, selection of an appropriate analytic method is important for quantification of acetaldehyde in alcoholic beverages.

Keywords Acetaldehyde · Alcoholic beverage · Sulphite · Wine

Introduction

According to the World Health Organization, annual alcoholic beverage consumption per person aged 15 years and older is 6.2 L worldwide, and Koreans consume 12.3 L annually and are ranked 15th (WHO, 2014). Alcoholic beverages produced in Korea in 2016 were beer (45.2%), soju (32.5%), takju (11.2%), spirit (7.9%), liqueur (1.0%), wine (0.8%), chungju (0.6%), and yakju (0.3%) (MFDS, 2016a). Yakju is made by clear filtration of rice-wine mash obtained by fermentation of starch or vegetable materials and koji (MFDS, 2016a). Diluted soju is made by diluting spirit (95% ethyl alcohol called jujeong) produced by distillation of fermented starch or sugar containing materials (Lee et al., 2014; MFDS, 2016a). Fruit wine is produced mainly by fermenting fruits and fruit juice or by adding fruits, sugar, or carbonic acid during the fermentation process (MFDS, 2016a). In Korea, black raspberry wine, mulberry wine, and omija wine are sold on the market.

The quality of alcoholic beverages in Korea is controlled by regulating the amounts of ethanol, methanol, total acidity, preservatives, or aldehyde depending on the type of alcoholic beverage (MFDS, 2016b). Total acidity includes the total contents of volatile acids and organic acids and is used as an index for quality control or early determination of the rancidity of an alcoholic beverage (Song and Park, 2003). Acetaldehyde is formed biologically by yeast metabolism through decarboxylation of pyruvate (an intermediate in glycolytic pathway) during an early stage of an alcoholic fermentation, and chemically by an oxidation of ethanol by alcohol dehydrogenase (ADH) (Jackowetz et al., 2011). In addition, it is also partially generated by decayed microorganisms or low quality yeast (Chung et al., 2012). Upon ingestion, acetaldehyde is decomposed into acetic acid by aldehyde dehydrogenase

✉ Jeung-Hee Lee
jeunghlee@daegu.ac.kr
Kwang-Seup Shin
botomak2@naver.com

¹ Department of Food and Nutrition, Daegu University, 201 Daegudae-ro, Gyeongsan-si, Gyeongsangbukdo 38453, Republic of Korea

(ALDH) in the body, but excessive acetaldehyde hinders the ALDH reaction, accumulates, and then moves onto other organs through the blood to inhibit the function of mitochondria, resulting in hepatitis and liver cirrhosis (Park et al., 2006). Acetaldehyde is a Group 2B carcinogen, whereas acetaldehyde obtained by consumption of alcoholic beverages is classified as a Group 1 carcinogen (IARC, 1999). The regulatory concentration of acetaldehyde is 10 mg/L in spirits and 70 mg/L in distilled liquors such as soju, whisky, and brandy in Korea (MFDS, 2016a). Acetaldehyde content is usually analyzed by titration, headspace-GC, and enzymatic methods (Jackowetz et al., 2011; Lee, 2014). Titration methods are widely used in Korea (MFDS, 2016b), Japan (NTA, 2017), and USA (AOAC, 2000), whereas EU requires the titration method specified by the International Organisation of Vine and Wine (OIV, 2017) for measuring acetaldehyde in wine.

Sulphite is an essential antioxidant and microbiological control agent in wine manufacturing processes. However, it is known to reduce the quality of taste, aroma, and color by bonding with acetaldehyde (Jackowetz et al., 2011), and causes side effects such as bronchoconstriction, headache, abdominal pain, vomiting, and dizziness (Kim et al., 2010). The regulatory concentration of sulphite in alcoholic beverages varies by country: < 0.350 g/kg in Korea (MFDS, 2016a), < 150–400 mg/L in EU depending on the type of wine (EC, 2009), and < 350 mg/L in USA (CFR, 2017).

In this study, major commercial alcoholic beverages, including yakju, diluted soju, and fruit wines, distributed in the Korean market were collected to assess their qualities. Acetaldehyde contents of alcoholic beverages were quantified and compared by titration method, headspace-GC, and enzymatic assay. For fruit wine, titration method specified by OIV was added to these three methods to compare acetaldehyde quantification. In addition, pH level, total acidity, sulphite, and flavonoid content were analyzed to evaluate the quality characteristics of the alcoholic beverages.

Materials and methods

Materials

A total of five types of commercial alcoholic beverages on the market, including yakju (sixteen brands, Korea), diluted soju (two brands, Korea), plum wines (three brands, Korea), red wine (two brands, USA and Chile), and white wine (five brands, Chile, USA, Australia, and Argentina) were purchased from a nearby wholesale supermarket. Yakju as Korean traditional alcoholic beverages were divided into sterilized and non-sterilized yakjus, and their qualities were compared, and the quality of plum wine was

compared with those of red and white wines. Diluted soju was used as a control. Activated charcoal, polyvinylpyrrolidone (PVPP), 0.5 M iodine solution (I_2), 0.1 M sodium thiosulfate solution ($Na_2S_2O_3$), aluminum chloride ($AlCl_3$), sodium nitrite, quercetin, ethyl alcohol, sodium nitroferrocyanide ($Na_2[Fe(CN)_5NO] \cdot 2H_2O$), piperidine ($C_5H_{11}N$), boric acid, and acetaldehyde were purchased from Sigma Aldrich CO., Ltd (St. Louis, MO, USA). Sodium hydrogen sulfite ($NaHSO_3$), soluble starch, and 1 M sodium hydroxide solution were obtained from Daejung Chemicals & Metals CO., Ltd (Siheung-si, Gyeonggi-do, Korea). Phenolphthalein and 0.1 M NaOH solution were bought from Samchun Pure Chemical CO., Ltd (Pyeongtaek city, Gyeonggi-do, Korea), and citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) was bought from DC Chemical CO., Ltd (Seoul, Korea).

Measurement of pH and total acidity of alcoholic beverages

The pH level was measured repeatedly four times using a pH meter (Orion 420A+, Thermo electron Co., Beverly, MA, USA). For analysis of total acidity (TA), 200 mL of boiled and chilled distilled water was placed into a beaker and titrated to pH 8.2 with 0.1 M NaOH solution. Then, 5 mL of sample was added and titrated again to pH 8.2, and the TA (g/100 mL) of alcoholic beverages was calculated by the AOAC method (2000). The TA of each alcoholic beverage was represented as acetic acid (diluted soju and yakju), or tartaric acid (fruit wine).

Analysis of flavonoid contents in alcoholic beverages

Distilled water (4 mL) and 1 mL of diluted wine sample (10 times with distilled water) were mixed and left for 5 min, after which 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ were added, vortexed, and reacted for 6 min. The reactant solution was mixed with 2 mL of 1 M NaOH and 2.4 mL of distilled water, and the absorbance was measured at 510 nm with UV/VIS Spectrophotometer (Optizen 2120UV, Mecasys Co., Ltd, Daejeon, Korea) (Kang et al., 2009). Standard solutions of quercetin (0, 50, 100, 250, 500, 1000, 3000 mg/L) were prepared to obtain a calibration curve, and the flavonoid contents were expressed as mg of quercetin/L of alcoholic beverages.

Pre-treatment of fruit wine

White wine (50 mL) containing carbonic acid was placed in a beaker and stirred for 2 min at 200 rpm and for 3 min at 500 rpm to remove carbonic acid before being used in the analysis. For acetaldehyde quantification with the titration method, 2 g of activated charcoal was put into

25 mL of red wine, or white wine, mixed for 2–3 s, and left for 2 min, followed by filtration through filter paper before being used. For acetaldehyde quantification with enzymatic analysis, 25 mL of each wine was mixed with 0.5 g of PVPP and stirred for 5 min at 240 rpm in a stirrer and filtered before being used.

Method validation

Stock solution of acetaldehyde (10,000 mg/L in 15% ethanol) was used to prepare standard working solutions in the range of 5–500 mg/L. A method validation was evaluated by precision and accuracy. For precision validation of acetaldehyde quantification, the calibration curve was obtained with standard acetaldehyde solution with four different methods, and the linear regression and correlation coefficient (R^2) were presented. Accuracy of acetaldehyde quantification was assessed by recovery test with yakju or diluted soju spiked with 25 or 250 mg/L of acetaldehyde. To assess the effect of quercetin on a quantitative analysis of acetaldehyde in alcoholic beverages, recovery test was performed with acetaldehyde standard solution (50 and 100 mg/L in 15% ethanol solution) by mixing quercetin (10, 25, 50, 100 mg/L).

Analysis of acetaldehyde content with titration method

Sample (5 mL) and 45 mL of distilled water were put into a beaker, after which 0.01 N NaHSO_3 corresponding to 10 mL of 0.005 M I_2 was added and left for 30 min (MFDS, 2016a). Then, 10 mL of 0.005 M I_2 and 1 mL of 1% starch solution were added into the solution and titrated with 0.01 M $\text{Na}_2\text{S}_2\text{O}_3$ (A mL). For blank analysis, 5 mL of distilled water was also titrated (B mL). The acetaldehyde content of each alcoholic beverage was calculated by the following equation.

$$\text{Acetaldehyde (mg/L)} = \frac{(A - B) \times F \times 0.22 \times 1000}{\text{Test sample (mL)}}$$

A: 0.01 M $\text{Na}_2\text{S}_2\text{O}_3$ titration for sample (mL); B: 0.01 M $\text{Na}_2\text{S}_2\text{O}_3$ titration for blank (mL); F: 0.01 M $\text{Na}_2\text{S}_2\text{O}_3$ factor.

Analysis of acetaldehyde content with enzymatic assay

Acetaldehyde content was analyzed using acetaldehyde assay kits (K-ACHYD, Megazyme Company, Wicklow, Ireland). Two mL of distilled water and 0.1 mL of sample were placed into a cuvette, after which 0.2 mL each of buffer (pH 9.0) and NAD⁺ solution were added, mixed, and left for 2 min. The absorbance was measured at

340 nm using a UV/VIS Spectrophotometer (Optizen 2120UV, Mecasys Co., Ltd, Daejeon, Korea). Then, 0.05 mL of aldehyde dehydrogenase solution was added, mixed, and left for 3–4 min, and the absorbance was measured again at 340 nm. Acetaldehyde content of each alcoholic beverage was calculated using the formula provided by the assay kit.

Analysis of acetaldehyde content with headspace-gas chromatography

Acetaldehyde content was analyzed using headspace autosampler (HTA 2100, YL Instrument Co., Ltd, Anyang, Korea) and a gas chromatography (7890B GC system, Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector. The column was DB-Wax (30 m $\times$ 0.25 mm $\times$ 0.25 μm , Agilent Technologies, Santa Clara, CA, USA), and the carrier gas was N_2 (0.7 mL/min). Sample (5 mL) was diluted with 15% ethanol solution in an equal volume. Ten mL of the diluted sample was placed into a 20 mL vial (Cronus, Gloucester, UK) provided with a Sil/PTFE speta in the cap and incubated at 70 °C for 10 min to reach equilibrium of headspace. The conditions of headspace auto-sampling system are: headspace syringe temperature 75 °C; agitation during incubation on time 0.5 min; agitation during incubation off time 0.5 min; injection volume 1000 μL ; sampling speed 6.0 mL/min; injection speed 30 mL/min. The headspace (1000 μL) was injected into the GC at a split ratio of 20:1. Carrier gas was nitrogen with a constant flow of 0.7 mL/min. The temperatures of the injector and detector were set to 200 °C and 250 °C, respectively. The temperature of the oven was maintained at 35 °C for 5 min, raised to 80 °C at a rate of 3 °C/min and then to 250 °C at a rate of 10 °C/min, and maintained at 250 °C for 10 min. Standard solutions of acetaldehyde (10, 20, 50, 100, 200, 500, and 1000 mg/L in 15% ethanol) were prepared to obtain an external calibration curve, and the acetaldehyde contents were calculated and expressed as mg/L of alcoholic beverages.

Analysis of acetaldehyde contents in fruit wines with OIV titration method

Acetaldehyde contents of fruit wines were analyzed with the OIV method (OIV, 2017). Filtered wine (2 mL) was placed into an Erlenmeyer flask, after which 5 mL of 0.4% sodium nitroferricyanide solution and 5 mL of 10% piperidine solution were added to the wine. The mixture was moved to a cuvette immediately thereafter, and the absorbance was measured at 570 nm. Standard solution was prepared by combining acetaldehyde and sulfur dioxide (sulphite, SO_2) according to the method OIV-MA-

AS315-01, and calibration curves were prepared (40, 60, 120, 160, and 200 mg/L of acetaldehyde) to calculate the acetaldehyde contents of alcoholic beverages (mg of acetaldehyde/L of wine).

Analysis of total sulphite and free sulphite contents in alcoholic beverages

The contents of total sulphite and free sulphite contained in alcoholic beverages were analyzed with total sulphite assay kits (K-TSULPH) and total and free sulphite assay kits, respectively (K-SULPH) (Megazyme Company, Wicklow, Ireland). Analysis was performed according to the experimental protocol of the assay kit manual, and sulphite content was obtained by measuring the absorbance at 405 nm using the UV/VIS Spectrophotometer. Calibration curves were prepared using the standards provided in the assay kits, and sulphite content was calculated and expressed as mg of free sulphite (or total sulphite)/L wine.

Statistical analysis

The experimental results were expressed as the mean $\pm$ standard deviation. Analysis of variance (ANOVA) was performed using the Statistical Analysis System (SAS) 9.2 (SAS Inst. Inc., Cary, NC, USA), and statistical difference was determined by Duncan's multiple range test at $p < 0.05$.

Results and discussion

pH level, total acidity, and total flavonoid contents of commercial alcoholic beverages

pH level and total acidity (TA) are commonly used as indicators to evaluate the quality characteristics or rancidity of alcoholic beverages. When TA is high, progression of abnormal fermentation can be predicted, and when the TA is low, unique sourness may be lost, which may affect the flavor and preservability of alcoholic beverages (Moon et al., 2015; Song and Park, 2003).

The pH levels were 7.69–7.80 in diluted soju, and 3.78–4.69 in yakju (sterilized) and yakju (non-sterilized), and 2.91–3.57 in fruit wines. Thus, pH levels of diluted soju were the highest, and the pH level of fruit wine was the lowest (Table 1). Since diluted soju is manufactured by diluting spirit with water and then adding food additives such as sweeteners, its pH level is higher than those of other alcoholic beverages due to non-production of organic acids. The pH levels were reported as 3.89–4.58 in yakju (Lee et al., 2007), 3.30–3.90 in plum wine (Miljić et al., 2017; Satora and Tuszyński, 2010), 3.10–3.50 in red wine

(Yoo et al., 2010), and 3.06–3.76 in white wine (Yoon et al., 2016), which are similar to the results of the present study.

The TA levels were 0.21–0.73 g/100 mL in yakju, whereas TA was as high as 0.48–0.99 g/100 mL in fruit wines but not detected in diluted soju. Especially, TAs of yakju were lower than 0.7% (acetic acid), which are the regulatory TA limits in Korea (MFDS, 2016a). Major organic acids in alcoholic beverages are citric, malic, succinic, lactic, and acetic acid, which are produced by yeast during the fermentation process, and their contents vary depending on the type of alcoholic beverage (Moon et al., 2015; Yoo et al., 2008, 2016). The TA of yakju was reported as 0.40–0.56% (Huh et al., 2012), and the TA of yakju prepared with medicinal herbs increased as addition of medicinal herbs increased (Jin et al., 2008). The TA levels of wines mostly range from 0.5 to 1.0% (Vine, 1997) and are affected by grape variety, harvest time, and brewing method (Yoon et al., 2016). TA adjusted to 0.65% in red wine and to 0.75% in white wine provides freshness and a stable color and can suppress growth of spoilage bacteria (Anderson and Anderson, 1989; Kim et al., 2012). It has been reported that the TAs of imported red wines distributed in Korea are 0.5–0.6% while the TAs of domestic red wines are 0.4–0.8% (Chang et al., 2008).

Total flavonoid contents were as follows: red wine > white wine > plum wine > yakju. Red wine contained 10–30 times more flavonoids than white wine or plum wine, and no flavonoid was detected in other alcoholic beverages. Flavonoids such as catechin, quercetin, and kaempferol were detected in red wine (Lee et al., 2013), quercetin and catechin in white wine [30] as well as peonidin, cyanidin, and rutin in plum wine (Miljić et al., 2017).

Method validation

The method validation for precision was presented with a linear regression, and the four methods showed excellent linearity with the correlation coefficients (R^2) in the range of 0.9998–1.000 (Table 2). The accuracy of the methods was presented with recovery rate in the range of 92.8–98.7% with titration, 103.8–110.2% with enzymatic assay, 93.3–103.8% with HS-GC method (Table 2). The validation results for acetaldehyde quantification showed the acceptable performance of the methods done in present study, and the performance was lower in titration method than enzymatic and HS-GC methods.

Table 1 pH level, total acidity, and total flavonoid contents of commercial alcoholic beverages

Alcoholic beverages	Sample code	Alcohol (%)	pH	Total acidity ⁱ (g/100 mL)	Total flavonoid (mg/L)
Diluted soju	DUS-1	17.8	7.69 ± 0.01 ^b	ND	ND
	DUS-2	17.8	7.80 ± 0.01 ^a	ND	ND
Yakju (sterilization)	YS-1	13.0	3.78 ± 0.00 ^h	0.53 ± 0.01 ^a	ND
	YS-2	13.0	3.86 ± 0.00 ^g	0.37 ± 0.00 ^b	ND
	YS-3	13.0	4.16 ± 0.00 ^f	0.22 ± 0.01 ^e	3.00 ± 0.79 ^e
	YS-4	16.5	4.65 ± 0.00 ^b	0.21 ± 0.00 ^e	100.22 ± 1.57 ^a
	YS-5	17.0	4.69 ± 0.00 ^a	0.22 ± 0.01 ^e	19.67 ± 0.79 ^d
	YS-6	13.0	4.51 ± 0.00 ^c	0.22 ± 0.01 ^e	26.33 ± 2.36 ^c
	YS-7	15.0	4.49 ± 0.00 ^d	0.24 ± 0.01 ^d	44.67 ± 1.57 ^b
	YS-8	13.0	4.38 ± 0.00 ^e	0.31 ± 0.00 ^c	42.44 ± 3.14 ^b
Yakju (non-sterilization)	YNS-1	15.0	4.39 ± 0.00 ^b	0.38 ± 0.00 ^d	45.78 ± 3.14 ^c
	YNS-2	15.0	4.09 ± 0.00 ^f	0.36 ± 0.00 ^d	19.67 ± 0.79 ^f
	YNS-3	17.0	4.36 ± 0.00 ^c	0.73 ± 0.01 ^a	84.67 ± 1.57 ^a
	YNS-4	15.0	4.05 ± 0.00 ^g	0.40 ± 0.00 ^c	45.78 ± 1.57 ^c
	YNS-5	14.0	4.50 ± 0.00 ^a	0.27 ± 0.00 ^e	36.33 ± 2.36 ^d
	YNS-6	15.0	4.14 ± 0.00 ^e	0.47 ± 0.02 ^b	56.89 ± 1.57 ^b
	YNS-7	12.0	4.20 ± 0.00 ^d	0.27 ± 0.01 ^e	28.00 ± 1.57 ^e
	YNS-8	12.0	3.98 ± 0.00 ^h	0.25 ± 0.00 ^f	20.22 ± 1.57 ^f
Plum wine	FP-1	14.0	3.11 ± 0.00 ^b	0.55 ± 0.00 ^c	149.11 ± 1.57 ^a
	FP-2	14.0	3.22 ± 0.00 ^a	0.67 ± 0.01 ^b	138.00 ± 0.00 ^b
	FP-3	10.0	2.91 ± 0.00 ^c	0.85 ± 0.05 ^a	106.33 ± 2.36 ^c
Red wine	FR-1	11.0	3.47 ± 0.00 ^b	0.99 ± 0.01 ^a	3015.78 ± 31.43 ^b
	FR-2	13.0	3.57 ± 0.00 ^a	0.50 ± 0.04 ^b	3860.22 ± 31.43 ^a
White wine	FW-1	13.5	3.45 ± 0.00 ^a	0.62 ± 0.01 ^a	393.56 ± 1.57 ^a
	FW-2	11.0	3.20 ± 0.00 ^d	0.48 ± 0.21 ^a	208.56 ± 2.36 ^c
	FW-3	6.0	3.38 ± 0.00 ^b	0.62 ± 0.01 ^a	90.78 ± 0.79 ^e
	FW-4	12.0	3.17 ± 0.00 ^c	0.69 ± 0.01 ^a	104.11 ± 0.79 ^d
	FW-5	13.0	3.23 ± 0.00 ^c	0.66 ± 0.00 ^a	257.44 ± 0.79 ^b

ND Not detected

^{a–h}Means of same type of alcoholic beverages with different superscript in the same column are significantly different by Duncan's multiple range test at $p < 0.05$ ⁱTotal acidity (as acetic acid in diluted soju, and yakju as well as tartaric acid in plum, and red and white wines) analyzed by the AOAC method

Comparison of acetaldehyde quantification of yakju and diluted soju

The aldehyde content in soju, whisky, brandy, and general distilled spirits is regulated to be below 70 mg/L in Korea (MFDS, 2016a). In this study, the acetaldehyde contents of alcoholic beverages were analyzed using three methods (titration, enzymatic assay, and HS-GC) in order to verify that acetaldehyde contents were below regulation limits as well as determine any significant differences among the analytical methods (Table 3).

Diluted soju is manufactured by diluting fermented spirit with water and adding additives, in which the spirit (almost 95% ethyl alcohol) is produced at high purity by fermentation of starch and continuous distillation (Lee et al., 2012). Since acetaldehyde is reduced during the

distillation process, diluted soju is considered to have lower acetaldehyde content than other alcoholic beverages (Lee et al., 2012). The acetaldehyde content of sterilized yakju was the highest as measured by titration (excluding YS-4) ($p < 0.05$), whereas the acetaldehyde content of non-sterilized yakju varied by the type of yakju and analytic method. The amount of acetaldehyde varied since yakju is prepared by addition of various plant materials such as medicinal herbs (ginseng, pine needles, etc.) during the fermentation process. The previous study reported that commercial yakju contains an acetaldehyde content of 2.88–11.76 ppm (Park et al., 2006), and yakju made with fresh ginseng, white ginseng, and red ginseng has acetaldehyde contents of 23.48 ppm, 13.09 ppm, and 9.60 ppm, respectively (Roh et al., 2001). And acetaldehyde content decreased from 19.98 to 17.73 mg/L at high

Table 2 Comparison of linear regressions and recovery rate of acetaldehyde by different analytic methods

	Linear regressions			
	Titration ^a	Enzymatic ^b	HS-GC ^c	OIV ^d
Calibration curve	$y = 0.8806x + 3.1868$	$y = 0.8675x + 1.2401$	$y = 2.9363x - 0.1636$	$y = 0.0049x + 0.0225$
Correlation coefficient (R^2)	0.9997	0.9998	1.0000	0.9999
Calibration range (mg/L)	5–250	5–250	5–500	5–200
	Recovery rate (%)			
	Titration	Enzymatic	HS-GC	
<i>Yakju</i>				
+ 25 mg/L	98.7 ± 1.3	103.8 ± 1.5	103.8 ± 1.5	
+ 250 mg/L	94.7 ± 0.1	104.0 ± 0.2	102.5 ± 1.9	
<i>Diluted soju</i>				
+ 25 mg/L	98.7 ± 3.8	110.2 ± 0.5	100.8 ± 3.0	
+ 250 mg/L	92.8 ± 0.0	108.0 ± 0.4	93.3 ± 6.4	

^aModified titration method from Korean Food Standards Codex; plum, and red and white wines were pretreated with activated charcoal

^bAnalyzed by enzyme assay kit; plum, and red and white wines were pretreated with PVPP

^cHeadspace—gas chromatography

^dInternational organisation of vine and wine

Table 3 Comparison of acetaldehyde contents in commercial alcoholic beverages by different analytic methods

Alcoholic beverages	Sample code	Acetaldehyde (mg/L)		
		Titration ^e	Enzymatic ^f	HS-GC ^g
Diluted soju	DUS-1	0.44 ± 0.63 ^a	0.53 ± 0.25 ^a	0.64 ± 0.06 ^a
	DUS-2	1.33 ± 0.63 ^a	0.45 ± 0.13 ^{ab}	0.11 ± 0.00 ^b
Yakju (sterilization)	YS-1	19.56 ± 1.26 ^a	16.40 ± 0.76 ^b	14.35 ± 0.29 ^b
	YS-2	21.78 ± 0.63 ^a	19.88 ± 0.88 ^a	16.39 ± 0.29 ^b
	YS-3	18.67 ± 1.26 ^a	15.96 ± 0.63 ^b	14.72 ± 0.14 ^b
	YS-4	16.89 ± 1.26 ^b	22.91 ± 1.39 ^a	19.59 ± 0.39 ^{ab}
	YS-5	36.44 ± 0.00 ^a	32.81 ± 0.00 ^{ab}	30.25 ± 2.36 ^b
	YS-6	24.00 ± 1.26 ^a	13.82 ± 0.38 ^b	15.30 ± 0.10 ^b
	YS-7	20.89 ± 0.63 ^a	13.46 ± 0.13 ^b	14.04 ± 0.05 ^b
	YS-8	14.67 ± 0.63 ^a	9.90 ± 0.13 ^b	7.81 ± 0.39 ^c
Yakju (non-sterilization)	YNS-1	9.33 ± 1.89 ^b	23.62 ± 0.63 ^a	23.44 ± 2.07 ^a
	YNS-2	14.67 ± 0.63 ^b	18.28 ± 0.63 ^a	17.45 ± 0.05 ^a
	YNS-3	16.44 ± 0.63 ^a	8.11 ± 0.63 ^b	7.30 ± 0.14 ^b
	YNS-4	5.78 ± 0.63 ^b	10.34 ± 0.50 ^a	11.28 ± 0.19 ^a
	YNS-5	10.22 ± 0.53 ^b	22.91 ± 0.88 ^a	24.09 ± 0.87 ^a
	YNS-6	8.89 ± 0.00 ^b	19.61 ± 0.50 ^a	20.14 ± 0.48 ^a
	YNS-7	27.56 ± 1.26 ^a	16.58 ± 0.76 ^b	14.52 ± 0.05 ^b
	YNS-8	41.33 ± 0.63 ^a	23.36 ± 1.26 ^b	23.95 ± 0.48 ^b

^{a–c}Means with different superscript in the same row are significantly different by Duncan's multiple range test at $p < 0.05$

^eModified titration method from Korean Food Standards Codex

^fAnalyzed by enzyme assay kit

^gHeadspace—gas chromatography

temperature during distillation (Min et al., 1997). Thus, it can be inferred that the added materials and manufacturing processes influence the acetaldehyde content of yakju. The acetaldehyde contents of diluted soju, sterilized yakju, and non-sterilized yakju were detected to be below the regulation limit of Korean government (70 mg/L), and the content varied according to the type of alcoholic beverage.

Comparison of acetaldehyde quantification contents in fruit wines

Acetaldehyde contents of fruit wines were analyzed with the OIV method used in the EU as well as titration, enzymatic assay, and HS-GC (Table 4). The acetaldehyde contents of plum wine were in the following order: enzymatic > OIV > titration > HS-GC ($p < 0.05$). In red wine, acetaldehyde content was in the following order: OIV > titration > enzymatic > HS-GC. In white wine, acetaldehyde contents were in the following order: OIV > enzymatic > titration > HS-GC ($p < 0.05$).

In fruit wines, acetaldehyde content was highest in the order of OIV method and enzymatic assay > titration > HS-GC. Especially in white wine, titration and HS-GC methods showed remarkably lower acetaldehyde contents than other analytical methods. Since acetaldehyde in wine binds to SO_2 or reacts with flavonoids (catechin, anthocyanin, and tannin) forming polymers (ethyl-bridged condensation product) (Sheridan and Elias, 2016; Timberlake and Bridle, 1976), acetaldehyde can be detected in lower amounts by titration or GC analysis. The method

validation of acetaldehyde quantification in a standard solution containing both acetaldehyde and quercetin (as flavonoid) was compared (Table 5). The recovery rate of acetaldehyde with the enzymatic assay was 96.10–119.64% (at acetaldehyde of 50 mg/L), and 92.71–102.70% (at acetaldehyde of 100 mg/L) and while the recovery rate with the titration method was 51.56–88.89% and 73.78–93.33%, respectively. As the content of quercetin increased, the acetaldehyde quantification value by titration method decreased remarkably, thus the detection amount of acetaldehyde could be reduced by binding of acetaldehyde with flavonoid in alcoholic beverages.

Contents of total sulphite and free sulphite in commercial alcoholic beverages

The total sulphite (TSO_2) contents and free sulphite (FSO_2) contents of alcoholic beverages were measured, as shown in Table 6. Sulphite was detected only in fruit wines among the commercial beverages analyzed in present study. SO_2 was shown to exist in free or bound form in red and white wines, whereas SO_2 mostly existed in bound form in plum wine. TSO_2 content was highest in the order of white wine > red wine > plum wine, and FSO_2 content was also higher in white wine than in red wine. It has been reported that the TSO_2 and FSO_2 contents of domestic red wine were 8.00–45.00 mg/L and 3.00–12.00 mg/L, respectively (Roh et al., 2008), and the TSO_2 content of low-priced French red wine sold in Korea was reported to be 58.18–68.78 mg/L (Kim et al., 2010). White wines used in

Table 4 Comparison of acetaldehyde contents in commercial fruit wines by different analytic method

Alcoholic beverages	Sample code	Acetaldehyde (mg/L)			
		Titration ^e	Enzymatic ^f	HS-GC ^g	OIV ^h
Plum wine	FP-1	16.44 ± 0.63 ^c	34.77 ± 1.51 ^a	8.18 ± 0.14 ^d	30.49 ± 1.32 ^b
	FP-2	46.22 ± 1.26 ^b	54.02 ± 2.27 ^a	39.79 ± 0.53 ^c	49.98 ± 1.32 ^{ab}
	FP-3	23.11 ± 0.00 ^b	33.61 ± 0.38 ^a	18.57 ± 0.39 ^c	34.56 ± 1.08 ^a
Red wine	FR-1	33.33 ± 0.63 ^b	20.95 ± 0.38 ^c	5.90 ± 0.48 ^d	37.10 ± 0.60 ^a
	FR-2	21.33 ± 0.00 ^c	25.23 ± 0.63 ^b	5.90 ± 0.00 ^d	37.36 ± 1.20 ^a
White wine	FW-1	7.56 ± 0.63 ^c	68.64 ± 2.02 ^b	9.10 ± 0.29 ^c	79.81 ± 0.84 ^a
	FW-2	10.22 ± 0.63 ^c	37.53 ± 0.13 ^b	7.23 ± 0.05 ^d	48.46 ± 0.60 ^a
	FW-3	10.67 ± 0.00 ^c	56.25 ± 0.38 ^b	7.74 ± 0.00 ^d	83.20 ± 1.56 ^a
	FW-4	16.00 ± 0.00 ^c	39.85 ± 1.13 ^b	5.22 ± 0.48 ^d	49.73 ± 1.68 ^a
	FW-5	4.00 ± 0.63 ^d	39.58 ± 0.50 ^b	5.63 ± 0.10 ^c	47.86 ± 0.48 ^a

^{a–d}Means with the different superscript in same row are significantly different by Duncan's multiple range test at $p < 0.05$

^eModified titration method from Korean Food Standards Codex; plum, and red and white wines were pretreated with activated charcoal

^fAnalyzed by enzyme assay kit; plum, and red and white wines were pretreated with PVPP

^gHeadspace—gas chromatography

^hInternational organisation of vine and wine

Table 5 Recovery rate of acetaldehyde in standard solution containing quercetin with titration and enzymatic assay

Concentration		Recovery rate (%)	
Acetaldehyde (mg/L)	Quercetin (mg/L)	Titration ^a	Enzymatic ^b
50	0	88.89 ± 0.00*	96.10 ± 0.76
	10	83.56 ± 0.00*	100.92 ± 1.51
	25	80.89 ± 1.26*	105.55 ± 0.50
	50	65.78 ± 0.00*	109.12 ± 2.52
	100	51.56 ± 2.51*	119.64 ± 0.76
100	0	93.33 ± 0.00	92.71 ± 0.76
	10	91.11 ± 0.63	92.71 ± 0.25
	25	88.00 ± 0.00*	93.52 ± 0.38
	50	82.67 ± 1.26*	97.53 ± 0.00
	100	73.78 ± 0.00*	102.70 ± 0.00

*Means in same row are significantly different by student's *t* test at *p* < 0.05

^aModified titration method from Korean Food Standards Codex

^bAnalyzed by enzyme assay kit

Table 6 Contents of total and free sulphite in commercial fruit wines

Alcoholic beverages	Sample code	Sulphite (mg/L)	
		Total	Free
Plum wine	FP-1	23.10 ± 0.18 ^a	ND
	FP-2	9.85 ± 0.88 ^b	ND
	FP-3	4.48 ± 0.35 ^c	ND
Red wine	FR-1	26.60 ± 1.59 ^b	4.68 ± 0.00 ^b
	FR-2	45.98 ± 0.71 ^a	8.27 ± 1.06 ^a
White wine	FW-1	156.35 ± 0.53 ^b	36.93 ± 2.24 ^a
	FW-2	92.73 ± 0.00 ^c	11.85 ± 1.65 ^c
	FW-3	158.98 ± 0.71 ^a	33.10 ± 2.95 ^{ab}
	FW-4	140.48 ± 1.06 ^c	29.43 ± 1.06 ^b
	FW-5	112.98 ± 0.71 ^d	30.02 ± 0.47 ^b

ND not detected

^{a-e}Means of same type of alcoholic beverages with different superscript in the same column are significantly different by Duncan's multiple range test at *p* < 0.05

this study were imported (Chile, USA, Australia, and Argentina), and their TSO₂ contents were similar to those of Spanish white wine (82.10–147.20 mg/L) and lower than those of Korea white wine (165.78–166.96 mg/L), while their FSO₂ contents were lower than those of Spanish white wine (3.20–6.40 mg/L) (Koh and Lee, 1996; Mataix and Luque de Castro, 1998).

SO₂, added in the post-malolactic fermentation stage of wine, has antioxidant and anti-microbial effects only in free form; however it reacts with pigments, glucose, or carbonyl groups (diacetyl, galacturonic acid, pyruvic acid, α-ketoglutaric acid, and acetaldehyde) which are by-products from fermentation in wine, resulting in an inactive state of bound form. More than 80% of bound SO₂ in wine is reported to be combined especially with acetaldehyde (Jackowetz et al., 2011). SO₂ is added in a small amount to

red wine since red wine contains antioxidants such as proanthocyanidin, resveratrol, and flavonol from grape skins and seeds (Frankel et al., 1993; Yoo et al., 2004); whereas white wine is fermented after grape skins are removed, resulting in lowered antioxidant content than red wine. Thus, a large amount of SO₂ is added for production of white wine. As the amount of SO₂ added increases in wine manufacturing, formation of acetaldehyde increases (Frvik and Ebeler, 2003; Somers and Wescombe, 1987). Since when the amount of SO₂ increases, activity of yeasts or lactic acid bacteria is inhibited and a reuse of acetaldehyde is decreased in the late stage of fermentation and malolactic fermentation, leading to an increased amount of acetaldehyde, and resulted in an increased amount of bound SO₂ with acetaldehyde and a lower detection amount in wines with HS-GC or titration method

(Jackowetz et al., 2011). In the case of sweet wines, SO₂ also binds to glucose, which requires a higher amount of SO₂ addition (Jackowetz et al., 2011). The addition amount of SO₂ in wine is regulated by each country as follows: < 350 mg/kg in Korea, USA, and Canada as well as < 150 and 200 mg/L for red and white wines (sugar amount < 5 g/L), respectively, and < 200 and 250 mg/L (sugar > 5 g/L), respectively, in Europe (EC, 2009; Jackowetz et al., 2011; MFDS, 2016a).

Effect of SO₂ on quantitative analysis of acetaldehyde in wine

Quantification of acetaldehyde in alcoholic beverages is affected by whether or not SO₂ has been added as well as the amount of SO₂ added. SO₂ in red and white wines exists in bound and free forms, and FSO₂ contents are 17.6–18.0% (red wine) and 12.8–26.6% (white wine) of TSO₂ (Table 6). In plum wine, SO₂ content is low and mostly exists in bound form, thus, considerable amounts of acetaldehyde in wines are bound to SO₂ (Table 6). When the amount of acetaldehyde in bound form is large, the quantitative result of acetaldehyde can be low in titration and HS-GC analysis (Table 4) since less acetaldehyde is collected into the headspace during the pre-treatment process for GC analysis, and acetaldehyde bound to SO₂ cannot form a complex of sodium bisulfate–acetaldehyde in the titration method, thus, after adding iodine solution and titrating acetaldehyde with sodium thiosulphate solution, the titrated volume is small, resulting in lower acetaldehyde contents by calculation. On the other hand, aldehyde content is measured as total acetaldehyde (sum of free and SO₂ bound acetaldehyde) in the enzymatic assay, and especially in OIV analysis, acetaldehyde in wines is prepared in bound form with SO₂ before quantitative analysis, and this bound form of acetaldehyde is measured, so acetaldehyde quantification in wines may be relatively higher in enzymatic assay and OIV method than in titration and HS-GC. In present study, acetaldehyde contents of red and white wines with a high amount of SO₂ were significantly higher in order of OIV > enzymatic > titration > HS-GC method ($p < 0.05$). Therefore, enzymatic assay or OIV method is suitable for quantitative analysis of acetaldehyde in fruit wine for which addition of SO₂ is allowed.

In conclusion, acetaldehyde contents were different according to the type of alcoholic beverage and analytic method of titration, enzymatic, HS-GC, and OIV. Especially, acetaldehyde contents in fruit wines were significantly different according to analytic method ($p < 0.05$). Acetaldehyde bound to added SO₂ in wines affected quantification, resulting in lower acetaldehyde contents using titration and HS-GC method than the enzymatic and

OIV methods. Therefore, for quantitative analysis of acetaldehyde in alcoholic beverages, an selection of an appropriate analytic method according to the type of alcoholic beverage is important since various raw materials used, substances added, and manufacturing process could affect quantification results of the used analytical methods.

Acknowledgements This research was supported by the Daegu University Research Grant, 2015.

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