Validation and Determination of the Contents of Acetaldehyde and Formaldehyde in Foods

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(Received August 17, 2015; Revised September 17, 2015; Accepted September 21, 2015)

The aim of this study was to develop an efficient quantitative method for the determination of acetaldehyde (AA) and formaldehyde (FA) contents in solid and liquid food matrices. The determination of these compounds was validated and performed using gas chromatography-mass spectrometry combined by solid phase micro-extraction after derivatization with O-(2,3,4,5,6-pentafluoro-benzyl)-hydroxylamine hydrochloride. Validation was carried out in terms of limit of detection, limit of quantitation, linearity, precision, and recovery. Then their contents were analyzed in various food samples including 15 fruits, 22 milk products, 31 alcohol-free beverages, and 13 alcoholic beverages. The highest contents of AA and FA were determined in a white wine (40,607.02 ng/g) and an instant coffee (1,522.46 ng/g), respectively.

Key words: Acetaldehyde (AA), Formaldehyde (FA), Validation, Food products, GC-MS, SPME

INTRODUCTION

Low molecular weight aldehydes such as acetaldehyde (AA) and formaldehyde (FA), which are contained in foods, have received a special attention due to their high toxicity and carcinogenicity (1). AA is naturally occurred in diverse foods such as fruits, vegetables, dairy products, and fruit beverages (2). In addition, it is also added as a flavor enhancer in various beverages including soft drink and as a preservative in fruits and fish products (2). AA provides a pleasant fruity aroma at low levels while it has an irritating odor note at high levels (2). In fruits, it is produced as an intermediate in the respiration of higher plants, whereas, in alcoholic beverages, it is mainly formed by yeasts, acetic acid bacteria, and the auto-oxidation of ethanol and phenolic compounds (3). In addition, AA can be produced from alanine metabolism by some yeasts (4). Its level can increase due to the chemical oxidation of ethanol during aging of spirits (5). AA is also generated from Strecker degradation of alanine in the food systems (6,7). Furthermore, AA, a saturated aldehyde, can be formed as one of secondary products from the lipid oxidation of polyunsaturated fatty acids through chemical and/or enzymatic reactions in food systems (8). AA is extremely reactive and binds readily to proteins, peptides, and amino acids (9). AA is also able to cross-link to proteins, suggesting that it can react with DNA which may cause further biological changes, including mutagenesis and carcinogenesis (2). In addition, AA can exacerbate the neurologic, hepatic, and cardiac complications of alcoholism and cause membrane damages and inhibition of several enzyme activities such as aldehyde dehydrogenase which causes metabolic strain to acetate (10). The International Agency for Research on Cancer (IARC) classified AA as possibly carcinogenic to humans (Group 2B) and AA associated with the consumption of alcoholic beverages as carcinogenic to humans (Group 1) (11).

FA is colorless, highly volatile, and flammable with a strong and irritating odor. It is readily soluble in water, alcohol, and other polar solvents. FA is commercially produced from methanol and used as a preservative, reducing agent, and a sterilizing agent in food industry (12). It is naturally present as a product of normal metabolism in many foods including fruits, vegetables, meats, fish, crustaceans, and dried mushrooms (12). In some sea foods and crustaceans,
FA is known to develop postmortem from the enzymatic reduction of trimethylamine oxide (TMAO) in their bodies to FA and dimethylamine (13). In this process, the level of FA is different among the species and between frozen and fresh seafood due to their different amount of TMAO from species to species and depending on bacterial activity (13). FA is also generated from the oxidation of dietary methanol or methanol derived from aspartame, an artificial sweetener (14). In addition, it can be formed from Strecker degradation of glycine in the presence of glyoxal in food system (15). Furthermore, FA, a saturated aldehyde, is also derived from the lipid oxidation of polyunsaturated fatty acids by chemical and enzymatic reactions in food systems (8). FA has been related to the increased risks of leukemia and nasopharyngeal cancer in humans (16). The classification of FA is carcinogenic to humans by IARC (Group 1) (17) and known to be a human carcinogen by the US National toxicology program (NTP) (18).

GC-electron capture detection (ECD), GC-mass spectrometry (MS), headspace (HS)-SPME GC system, and high performance liquid chromatography (HPLC) after derivatization are mostly used to determine AA and FA in foods (19). Since both are highly volatile and reactive to carbonyl compounds, they are usually required to derivatize prior to analysis. The most common derivatization reagents include 2,4-dinitrophenylhydrazine (DNPH) (20,21), PFBHA (22,23), and 2-aminoethanethiol (cysteamine) (1,24). Regarding analytical methods for AA and FA, GC system has higher sensitivity and selectivity for both compounds compared to HPLC system (23).

AA in various foods was determined using HS-GC-FID after the extraction using simulated digestion, and the limit of detection and the limit of quantification were 0.01 mg/L and 0.04 mg/L, respectively (25). In recent, AA in children foods including yogurt, purees, and milk products was analyzed using SPME-GC combined with time of flight (TOF)-MS after derivatization with PFBHA (26). In addition, AA level in alcoholic and non-alcoholic beverages was also evaluated using GC-MS combined with SPME after PFBHA derivatization (27). European Union (EU) has recommended that AA in alcoholic beverages such as spirits is analyzed using GC-flame ionization detection (FID) with a direct injection method (28). The content of FA in various fish species was evaluated using SPME-GC-MS system based on derivatization with PFBHA with LOD of 17 µg/kg and LOQ of 28 µg/kg (13). Its level in Korean traditional fermented foods including kimchi, soybean paste, and soy sauce was determined using HS-SPME-GC-MS after derivatization with 2,2,2-trifluoroacetoxyhydrazine (TFEH) (29). There is little information available on the levels of AA and FA in a variety of foods since their determination has been reported in only very limited food products such as alcoholic beverages, fermented foods and fish products (21, 29,30).

The objective of the current study was to determine the contents of both AA and FA in a variety of food groups consumed in Korea using SPME-GC-MS after derivatization with PFBHA.

**MATERIALS AND METHOD**

All chemicals used were of analytical grade. AA, FA, and AA 1,2-13C2 (an internal standard compound) were purchased from Cambridge Isotope Laboratories, Inc., (Andover, MA, USA) and derivatizing agent PFBHA [O-(2,3,4,5,6-pentafluoro-benzyl)-hydroxylamine hydrochloride] was obtained from Sigma Aldrich (St. Louis, MO, USA).

**Preparation of standard solutions and method validation.** Stock standard solutions of AA, FA and AA 1,2-13C2 were prepared at 10,000 mg/L in deionized water. All standard solutions were stored at −5°C before use. Validation was carried out in food matrices, such as peanut butter, beef, milk, 20% ethanol solution, rice porridge, orange juice, and corn oil. Calibration samples were prepared in the range of 5~10,000 ng/g using food matrices and standard solutions. Limit of detection (LOD) and limit of quantitation (LOQ) were defined as lowest concentration with signal-to-noise (S/N) ratios of 3.3 and 10, respectively.

**Sample preparation and solid phase micro-extraction.** All samples obtained were kept at −70°C until being used for the experiments. Solid and semi-solid sample (1 g) were mixed with 9,950 mL of 30% NaCl solution and an internal standard [50 µL AA 1,2-13C2 (10 µg/L, w/v)] and then sonicated at ambient temperature for 30 min. After the sample was centrifuged (3000 rpm) at 4°C for 10 min and vortexed for 30 sec, supernatant (5 mL) was transferred to in 10-mL headspace vials with screw caps. Being mixed with 4,475 mL of 30% NaCl solution and an internal standard [25 µL AA 1,2-13C2 (10 µg/L, w/v)], liquid sample was sonicated at ambient temperature for 30 min. Then 100 mg potassium hydrogen phthalate (KHP) and 50 µL PFBHA (10 mg/mL) were added to 5 mL sample before voltexing for 30 sec and derivatizing at 45°C for 40 min. SPME fiber coated with 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco, Bellefonte, PA, USA) was used to adsorb volatile compounds in the headspace at 45°C for 15 min.

**Analysis by gas chromatography-mass spectrometry (GC-MS).** GC-MS analysis was performed using a 7890A series gas chromatograph connected to a 5975C mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5MS column (30 m length × 0.25 mm i.d. × 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). Helium was run as a carrier gas at a constant column flow rate of 0.8 mL/min. In the case of
SPME, the fiber was maintained in the splitless mode in GC injector at 220°C for 5 min to desorb the adsorbed volatiles. GC oven temperature was held 50°C for 2 min, raised to 120°C at a rate 30°C/min and then held at 200°C for 10 min. The other GC-MS conditions were as follows: The front inlet and detector transfer line temperatures were 220°C and 250°C, respectively. Mass spectra were obtained at 70 eV through electron ionization (EI). Data were acquired in the selected-ion monitoring mode (SIM mode). AA, FA and AA 1,2-13C2 derivatives were quantified at m/z-209, 195, and 211, respectively. Additionally, 181 at m/z was monitored for qualifier ion of AA, FA and AA 1,2-13C2.

RESULT AND DISCUSSION

Method validation. An efficient method was developed to analyze the contents of AA and FA in various food matrices. Method validation included linearity, LOD, and repeatability of the present method using calibration samples spiked with authentic AA and FA compounds. Linearity (correlation efficient r) ranged from 0.949~0.9993 (Table 1). LOD ranged from 5.74~175.03 ng/g. On the other hand, RSD (%) of precision ranged from 1.34~14.53, whereas recovery (%) was in the range of 68.37~128.22%. Our validation results showed that the method had an acceptable performance for their analytical method based on Guidelines for the Validation of Chemical Methods for the FDA Foods Program.

Aldehydes contents in food samples. The results obtained from various food samples are presented in Table 2-5.

The content of AA in fruits was in the range of 483.42~19,530.53 ng/g, whereas that of FA was in the range of 116.90~356.73 ng/g. There was a previous study on AA content in some fruits. AA content in apple, grape, kiwi, orange and pineapple was 320~2,390 ng/g, 910~3,230 ng/g, 730~810 ng/g, 5,560~8,370 ng/g, and 630 ng/g, respectively (25). In the case of FA, its level was also different depending on fruits; 6,300~22,300 ng/g in apple, 22,400 ng/g in grape, and 9,200 ng/g in watermelon (31). The present results on AA content in apple and grape were similar to those of the previous studies (32). However, in the case of kiwi, orange, and pineapple, higher content of AA was determined in this study compared to the previous ones. Also, AA content of canned pineapple was shown to be 483.42 ng/g, which was lower than that of raw pineapple.

### Table 1. The equations and the correlation coefficients of aldehydes

| Matrices     | Calibration curve                  | Limit of detection (ng/g) | Calibration range (ng/g) | Linearity |
|--------------|------------------------------------|----------------------------|--------------------------|-----------|
| **Acetaldehyde** |                                     |                            |                          |           |
| Rice porridge | Y = 0.0031X − 0.7071               | 51.8                       | 50-10,000                | 0.976     |
| Orange juice | Y = 0.0029X + 0.2312               | 19.9                       | 50-10,000                | 0.935     |
| Corn oil     | Y = 0.0022X + 0.2199               | 66.8                       | 50-10,000                | 0.988     |
| Peanut butter| Y = 0.0024X + 2.0043               | 175.03                     | 250-10,000               | 0.977     |
| Beef         | Y = 0.0025X + 0.426                | 25.08                      | 100-5,000                | 0.982     |
| Milk         | Y = 0.0023X − 0.0535               | 5.74                       | 50-10,000                | 0.993     |
| 20% EtOH     | Y = 0.0021X + 0.287                | 12.57                      | 50-10,000                | 0.973     |
| **Formaldehyde** |                                   |                            |                          |           |
| Rice porridge| Y = 0.0038X − 0.2777               | 38.0                       | 50-1,000                 | 0.950     |
| Orange juice | Y = 0.0029X − 0.0333               | 17.2                       | 50-1,000                 | 0.995     |
| Corn oil     | Y = 0.0073X − 0.7389               | 120.9                      | 50-1,000                 | 0.949     |
| Peanut butter| Y = 0.0066X + 0.1529               | 15.05                      | 50-1,000                 | 0.9921    |
| Beef         | Y = 0.0033X + 0.1611               | 7.80                       | 5-700                    | 0.9985    |
| Milk         | Y = 0.0056X + 0.0595               | 12.20                      | 5-1,000                  | 0.9974    |
| 20% EtOH     | Y = 0.0027X + 0.0056               | 11.00                      | 10-1,000                 | 0.998     |

Aldehydes contents in fruits

| Samples               | Relative peak areas (mean ± SD) |
|-----------------------|---------------------------------|
| Acetaldehyde (ng/g)   | Formaldehyde (ng/g)             |
| Nectarine             | 4,570.57 ± 344.26               | 159.11 ± 2.07              |
| White peach           | 5,556.68 ± 360.50               | 131.05 ± 2.04              |
| Melon                 | 19,530.53 ± 700.84              | 356.73 ± 19.49             |
| Yellow Peach          | 7,304.45 ± 272.09               | 116.90 ± 1.60              |
| Water Melon           | 18,781.94 ± 792.77              | 122.95 ± 1.31              |
| Orange                | 11,219.61 ± 247.22              | 136.59 ± 6.61              |
| Plum                  | 4,110.92 ± 64.46               | 118.35 ± 5.12              |
| Oriental melon        | 14,187.56 ± 994.92             | 208.11 ± 8.45              |
| Campbell early grape  | 2,880.46 ± 121.40              | 352.72 ± 34.58             |
| Kyoho grape           | 4,926.49 ± 126.34              | 154.57 ± 2.84              |
| Green Apple           | 1,524.70 ± 64.71               | 133.12 ± 4.20              |
| Asian pear            | 2,633.63 ± 161.77              | 133.71 ± 1.21              |
| Kiwi                  | 2,318.86 ± 99.76               | 227.58 ± 6.07              |
| Pineapple             | 11,864.79 ± 361.70             | 129.30 ± 2.58              |
| Pineapple (canned)    | 483.42 ± 13.50                | 173.71 ± 5.52              |

*Average of relative peak areas to that of the internal standard (n = 3) ± standard deviation.*
This result was consistent with that of a previous literature on the comparison of AA contents between canned and raw carrot samples (25). In addition, FA contents of all fruits studied in this study were lower than those of the previous studies. In dairy products, the overall content of AA was N.D–1,525.18 ng/g, whereas that of FA was 26.67–409.74 ng/g. A previous study showed that AA contents were 2,400–3,300 ng/g and 3,400–4,500 ng/g, respectively (31). FA in alcoholic beverage is thought to be mainly generated by bacteria that oxidize methanol (23). Glycine is also converted to acetaldehyde and formaldehyde depending on the types of beverages; 1,350–9,860 ng/g (in beer, 20,061.48 ng/g, whereas that of FA was 43.50 ng/g, which was not increased compared to that of raw milk. Also, the content of FA in processed milk was 43.50 ng/g, in the range of natural FA content suggested by WHO. In the present study, the content of FA can be increased by fermentation and thermal processing in dairy products. In the present study, the content of FA in yogurts and cheeses was similar to that of previous researches. On the other hand, the content of FA in raw milk was shown to be 42.88–54.05 ng/g, which was in the range of natural FA content suggested by WHO. Also, the content of FA in processed milk was 43.50 ng/g, which was not increased compared to that of raw milk.

In alcohol-free beverages, the content of AA was N.D–1,525.18 ng/g, whereas that of FA was 26.67–409.74 ng/g. There have been some studies on the content of AA in various beverages. Different levels of AA were found depending on the types of beverages; 1,350–9,860 ng/g (in tea), 930–1,630 ng/g (in fruit drinks), 280 ng/g (in soft drinks), 10–5,890 ng/g (in fresh fruit juices), and 150–16,300 ng/g (in processed juices). On the other hand, instant coffee and roasted coffee were shown to contain 31,200–35,510 ng/g and 1,150–40,140 ng/g of AA (25).

Also, a previous study on FA content in beverages reported that FA content of processed soft drinks such as cola, fruit/vegetable juices, instant coffee, and roasted coffee were 7,400–8,700 ng/g, 800,000 ng/g, 10,000–16,000 ng/g, and 3,400–4,500 ng/g, respectively (31). FA in alcoholic beverage is thought to be mainly generated by bacteria that oxidize methanol (23). Glycine is also converted to FA by Strecker degradation (34). In the present study, AA content of tea was shown to be similar with those of previous studies whereas AA content of fruit drink was lower. We cannot detect AA in soft drinks, and previous studies

### Table 3. The contents of acetaldehyde and formaldehyde in dairy products

| Samples               | Acetaldehyde (ng/g) | Formaldehyde (ng/g) |
|-----------------------|---------------------|---------------------|
| Whole milk powder     | 77.71 ± 9.88        | 97.25 ± 1.33        |
| Nonfat dry milk       | 146.59 ± 11.04      | 128.62 ± 19.25      |
| Condensed milk        | 96.37 ± 6.71        | 134.14 ± 7.09       |
| Milk                  | N.D                 | 54.05 ± 6.86        |
| Low fat milk          | N.D                 | 42.88 ± 4.96        |
| Processed milk        | 162.78 ± 8.94       | 43.50 ± 4.71        |
| Ice cream cone        | 1,525.18 ± 133.71   | 276.63 ± 16.64      |
| Ice cream             | 276.45 ± 9.38       | 135.94 ± 14.60      |
| Ice cream (stick type)| 888.41 ± 32.57      | 346.81 ± 13.05      |
| Ice cream (cookie type)| 978.22 ± 34.42   | 409.74 ± 49.32      |
| Yogurt drink          | 423.12 ± 6.66       | 124.55 ± 3.82       |
| Semisolid yoghurt     | 7,331.38 ± 207.32   | 102.60 ± 0.42       |
| Liquefied yoghurt     | 6,898.63 ± 266.97   | 168.03 ± 9.25       |
| Cheese                | 175.72 ± 13.98      | 26.67 ± 0.83 (T)    |
| Mozzarella cheese     | 500.83 ± 35.97      | 57.17 ± 2.25        |
| Cheese stick          | 405.45 ± 56.98      | 182.28 ± 21.56      |
| Whipping cream        | 73.67 ± 10.65       | 42.07 ± 2.97        |
| Shrubet               | 855.69 ± 13.14      | 284.14 ± 23.48      |

*Average of relative peak areas to that of the internal standard (n = 3) ± standard deviation.

### Table 4. The contents of acetaldehyde and formaldehyde in alcohol-free beverages

| Samples                      | Acetaldehyde (ng/g) | Formaldehyde (ng/g) |
|------------------------------|---------------------|---------------------|
| Green tea (tea bag)          | 2,099.45 ± 187.10   | 577.77 ± 32.61      |
| Green tea powder             | 2,426.47 ± 170.28   | 669.30 ± 17.47      |
| Green tea based drinks       | 69.55 ± 3.44        | 125.28 ± 7.66       |
| Barley water                 | 483.40 ± 21.69      | 350.81 ± 6.83       |
| Sweet rice drink             | 174.54 ± 4.27       | 405.43 ± 33.46      |
| Coffee creamer               | 214.31 ± 0.67       | 452.58 ± 26.82      |
| Ground coffee                | 20,061.48 ± 96.51   | 269.95 ± 5.01       |
| Instant coffee 1             | 3,372.03 ± 428.23   | 1,522.46 ± 169.84   |
| Instant coffee 2             | 871.87 ± 40.99      | 445.64 ± 46.21      |
| Coffee (canned)              | 1,098.43 ± 21.07    | 267.53 ± 13.00      |
| Black coffee                 | 2,396.99 ± 55.86    | 415.66 ± 7.74       |
| Black coffee with sugar      | 2,293.21 ± 113.39   | 247.18 ± 9.09       |
| Coffee extract               | 1,012.75 ± 6.44     | 257.29 ± 12.89      |
| Sports drink                 | 159.13 ± 4.95       | 625.51 ± 46.22      |
| Sports drink (canned)        | 66.87 ± 0.83        | 467.79 ± 29.68      |
| Vitamin drink                | 425.07 ± 59.88      | 1,047.44 ± 128.07   |
| Aloe juice                   | 152.90 ± 3.24       | 798.85 ± 4.10       |
| Fruit drink 1                | 178.02 ± 2.94       | 550.03 ± 64.34      |
| Fruit drink 1 (canned)       | 915.69 ± 14.67      | 672.26 ± 45.15      |
| Fruit drink 2                | 152.74 ± 1.03       | 562.75 ± 9.32       |
| Fruit drink 2 (canned)       | 42.53 ± 0.29 (T)    | 523.15 ± 64.52      |
| Cola                         | N.D                 | 656.01 ± 11.99      |
| Cola (canned)                | N.D                 | 655.04 ± 72.56      |
| Sprite                       | N.D                 | 465.03 ± 4.11       |
| Sprite (canned)              | N.D                 | 568.73 ± 19.77      |
| Carrot juice                 | 234.25 ± 15.41      | 848.35 ± 14.97      |
| Soft drinks                  | 275.51 ± 1.27       | 637.19 ± 34.62      |
| Tomato juice                 | 315.76 ± 1.92       | 848.13 ± 18.84      |
| Fruit and vegetable juices   | 576.81 ± 16.86      | 809.25 ± 13.82      |
| Pear juice                   | 1,913.26 ± 5.30     | 713.71 ± 37.80      |
| Grape juice                  | 187.88 ± 8.36       | 212.20 ± 15.13      |

*Average of relative peak areas to that of the internal standard (n = 3) ± standard deviation.
Table 5. The contents of acetaldehyde and formaldehyde in alcoholic beverages

| Samples              | Relative peak areas (mean ± SD)* |
|----------------------|----------------------------------|
|                      | Acetaldehyde (ng/g) | Formaldehyde (ng/g) |
| Soju                 | 1,043.57 ± 15.99     | 86.48 ± 5.60        |
| Herb wine            | 11,940.98 ± 399.74   | 74.70 ± 5.12        |
| Chungju              | 10,195.97 ± 89.04    | 85.28 ± 7.85        |
| Makgeolli            | 40,506.43 ± 681.15   | 58.39 ± 0.67        |
| Apricot liqueur      | 20,778.63 ± 344.52   | 56.81 ± 5.47        |
| Beer (canned)        | 7,016.23 ± 122.93    | 45.47 ± 1.93        |
| Beer (PET bottled)   | 8,182.22 ± 259.55    | 28.20 ± 2.01(T)     |
| Imported beer (canned)| 4,152.42 ± 17.94     | 48.67 ± 2.75        |
| Bokbunjaju           | 8,680.25 ± 185.21    | 76.34 ± 5.08        |
| Red wine             | 17,323.72 ± 280.33   | 40.90 ± 2.69        |
| White wine           | 40,607.02 ± 159.36   | 18.38 ± 1.34(T)     |
| Sparkling wine       | 35,301.78 ± 923.13   | 19.50 ± 0.17(T)     |
| Whisky               | 29,301.17 ± 542.01   | 782.10 ± 6.10       |

*Average of relative peak areas to that of the internal standard (n = 3) ± standard deviation.

also have reported lower AA concentration in soft drinks than other beverages.

In alcoholic beverages, the content of AA was 1,043.57–40,607.02 ng/g, whereas that of FA was determined to be 18.38–782.10 ng/g. Previous studies on AA contents in alcoholic beverages showed that AA contents of red wine, white wine, sparkling wine, champagne, beer, whiskey, Makgeolli, Soju (Korean distilled spirits), Sake, and liqueur were 6,818–55,800 ng/g, 6,818–67,000 ng/g, 123,000ng/g, 2,355–8,460 ng/g, 76,900–15,263ng/g, 9,561ng/g, 805–13,371ng/g, 10,368ng/g, and 5,674–62,300ng/g, respectively (23,27). On the other hand, FA contents of wine, beer, whiskey, Makgeolli, Soju, Sake, and liqueur were 32 ng/g, 100–1,500 ng/g, 272ng/g, 60ng/g, 9–106ng/g, 27ng/g, and 228ng/g, respectively (23,33). AA is a highly volatile aroma component found in most beverages and foods. It provides a pleasant aroma like fruity note at low concentrations, whereas it can be related to a harsh odor note at high concentrations (2). Aldehydes, which are mainly produced by yeast during alcoholic fermentation, can be also generated by low-grade yeast and bacteria from non-fresh material during the manufacturing process of alcoholic beverage, affecting flavor characteristics of alcoholic beverages. AA is also very reactive, and can participate in binding with proteins via Schiff base. In particular, it can be easily lost by binding with amino groups of amino acids and peptides (2,3).

AA in alcoholic beverage, which had been previously considered as a by-product of the alcoholic fermentation by yeast, was also reported to be generated by the glucose metabolism of lactic acid bacteria, natural oxidation of ethanol in the presence of phenolic components (3), and the metabolism of alanine by yeasts (4). In the case of spirits, AA content was often increased by chemical oxidation of ethanol during aging and distillation processes (5). On the other hand, fermentation conditions of alcoholic beverage, such as the type of yeast, fermentation temperature, CO2 level, and raw material have been known to be highly involved in the generation of AA (30,33).

Analysis of AA content in alcoholic beverages showed that all samples, except for Makgeolli, contain lower level of AA, compared to that of previous studies. Since Makgeolli is not exposed to the filtration and sterilization process, AA content can be increased even after the ethanol fermentation by the continued metabolism of yeast. Particularly, AA content of Soju was lowest among alcoholic beverages studied. Most of fermentation by-products and flavor components are removed during the purification process (distillation). That purification process can be responsible for the lowest content of AA in Soju (23). The present results on FA content of alcoholic beverage indicated that all samples, except for Sake and whiskey, contain lower level of FA, compared to those of previous studies.

ACKNOWLEDGEMENTS

This research was supported by a grant (13162KFDA049) from Korea Food and Drug Administration in 2013–2015.

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