Quantitative Analysis of Acetaldehyde in Foods and Beverages

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Acetaldehyde in foods and beverages was analyzed by a newly developed derivatization method. Acetaldehyde was reacted with cysteamine to give 2-methylthiazolidine in a food or a beverage sample at pH 7 and room temperature. The 2-methylthiazolidine formed was extracted with dichloromethane and subsequently analyzed by a gas chromatograph equipped with a fused silica capillary column and a nitrogen-phosphorus detector. A total of 14 commercial food and beverage items were analyzed for acetaldehyde. The quantities of acetaldehyde in foods and beverages ranged from 0.46 (cola) to 101.9 ppm (whiskey). The results may be useful for risk assessment of acetaldehyde.

INTRODUCTION

Acetaldehyde is the second simplest aldehyde (next to formaldehyde). It is a colorless liquid that is volatile at room temperature and pressure. Pure acetaldehyde possesses a pungent irritating odor, but at dilute concentrations it gives a pleasant fruity aroma. It is widely used in such artificial fruit flavors as apple, apricot, banana, and peach (Arctander, 1969). Acetaldehyde is also commonly found in fruits such as citrus fruits because it is an intermediate product in the respiration of higher plants (Fishbein, 1979). It is also an intermediate product of alcohol fermentation and one of the sugar metabolites. In addition, aldehyde is formed by photochemical processes in the air (Finlayson-Pitts and Pitts, 1986) and is present in considerable amounts in automobile exhaust (California ARB, 1993). It is, therefore, one of the most abundant chemicals in foods and in the environment.

Acetaldehyde is extremely reactive and, due to its strong electrophilic properties (Donohue et al., 1983; Tuma and Sorrell, 1985), binds readily to proteins, the peptide glutathione, individual amino acids, and DNA. It has been reported (Lam et al., 1986) that acetaldehyde has the ability to cross-link to proteins in rat nasal respiratory mucosa both *in vitro* and *in vivo*, suggesting that acetaldehyde can react with DNA which may cause further biological changes, including mutagenesis and carcinogenesis.

In the present study, acetaldehyde in commercial foods and beverages was quantitatively analyzed by gas chromatography using a newly developed derivatization method.

MATERIALS AND METHODS

Materials. All analytical samples were purchased from local markets. Cysteamine hydrochloride and 2,4,5-trimethylthiazole were purchased from Aldrich Chemical Co., Milwaukee, WI. The standard stock solution of 2,4,5-trimethylthiazole was prepared by adding 10 mg of 2,4,5-trimethylthiazole to 1 mL of solvent and was stored at 5 °C. Authentic 2-methylthiazolidine was synthesized according to the method developed by Yasuhara and Shibamoto (1989a,b).

Analysis of Acetaldehyde in Various Commercial Foods and Beverages. *Sake, Beer, Soy Sauce, Whiskey, Apple Juice, Nonfat Milk, and Whole Milk.* Sake (150 mL), beer (150 mL), soy sauce (150 mL), whiskey (100 mL), apple juice (100 mL), nonfat milk (100 mL), and whole milk (50 mL) were diluted with deionized water to 200 mL in volume. Cysteamine hydrochloride (0.75 g) was added to each solution. The pH of the solution was immediately adjusted to 8 with 6 N NaOH. The solution was stirred for 30 min by a magnetic stirrer at room temperature. After the reaction mixture was adjusted to pH 7 with 5 N HCl, the solution was extracted with 50 mL of dichloromethane using a liquid-liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate for 10 h. After removal of sodium sulfate, the volume of the extract was adjusted to exactly 50 mL with dichloromethane. 2,4,5-Trimethylthiazole (100 μL) standard solution was added as an internal standard prior to GC analysis.

Instant Coffee, Roasted Coffee Beans, Instant Tea, Yogurt, Nonfat Yogurt, Green Tea, and Cocoa. Instant coffee (3 g), roasted coffee beans (3 g), instant tea (3 g), green tea (5 g), yogurt (50 g), nonfat yogurt (50 g), and cocoa (5 g) were each dissolved in 200 mL of deionized water, and then 0.75 g of cysteamine hydrochloride was added to each solution. Roasted coffee beans were gound into powder before use. The remaining steps of the procedure for each solution was the same as that for the sake except that the yogurt samples were stirred for 1 h.

Cola, Wines (Red, White, and Rosé), Orange Juice, and Root Beer. Cysteamine hydrochloride (0.75 g) was added to 200 mL each of cola, wine, orange juice, and root beer. The remaining steps of the procedure for each solution were the same as for the sake.

An aqueous solution (200 mL) of cysteamine (0.75 g) was treated by the same procedure as each experiment and the amount of acetaldehyde determined was used as blank for each sample. Each experiment was replicated three times.

An aqueous solution (200 mL) of 2-methylthiazolidine (10 μg) was extracted with 50 mL of dichloromethane at pH 7 to determine the efficiency of the extraction. The extraction was repeated three times.

Instrumental Analysis. A Hewlett-Packard (HP) Model 5890A gas chromatograph (GC) equipped with a nitrogen-phosphorus detector and a 30 m × 0.25 mm i.d. fused silica capillary column coated with DB-1 was used for quantitative analysis of 2-methylthiazolidine derived from acetaldehyde with cysteamine. The oven temperature was programmed from 60 to 180 °C at 4 °C/min and held for 10 min. Peak areas were integrated with HP 3390A series GC terminal. The injector and detector temperatures were 250 °C. The linear velocity of helium carrier gas was 30 cm/s.

RESULTS AND DISCUSSION

Even though acetaldehyde has been found in many foods and beverages—fruits, vegetables, bread, dairy products, meats, and alcoholic and nonalcoholic beverages (Maarse and Visscher, 1988)—there are only a few reports on quantitative analysis of acetaldehyde in foods and beverages. This may be due to the lack of a satisfactory analytical method for trace levels of acetaldehyde. Direct analysis of trace amounts of acetaldehyde by chromatographic methods is a task of considerable difficulty. Consequently, derivatization methods are commonly used. The most widely used derivative for chromatography is 2,4-dinitrophenylhydrazone (Reindl and Stan, 1982). The

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Table I. Results of Acetaldehyde Analysis in Foods and Calculated Amounts of Acetaldehyde Intake for Each Food When Consumed

food or beverage	amt acetaldehyde (ppm)	amt of food/serving	acetaldehyde intake/serving (mg)
	Group I		
sake A	60.2	375 mL/bottle	22.6
sake B	14.8	375 mL/bottle	5.54
sake C	23.5	375 mL/bottle	8.80
sake D	23.4	375 mL/bottle	8.76
sake E	36.0	375 mL/bottle	13.5
wine A	52.0	100 mL/glass	5.20
wine B	32.8	100 mL/glass	3.28
wine C	65.9	100 mL/glass	6.59
beer A	5.76	355 mL/can	2.05
beer B	11.7	355 mL/can	4.15
beer C	5.23	355 mL/can	1.86
whiskey A	59.3	100 mL/glass	5.93
whiskey B	25.0	100 mL/glass	2.50
whiskey C	102.0	100 mL/glass	10.2
apple juice	11.8	300 mL/glass	3.54
milk (nonfat)	0.82	180 mL/bottle	0.147
whole milk	1.65	180 mL/bottle	0.298
cola	0.461	354 mL/can	0.163
root beer	0.579	354 mL/can	0.205
orange juice	9.82	354 mL/can	3.48
yogurt (nonfat)	5.28	200 g/cup	1.06
yogurt	5.48	200 g/cup	1.10
soy sauce	4.33	*a*	*a*
	Group II		
instant coffee	1.09	1 g/180 mL/cup[b]	0.001
roasted coffee beans	1.08	3 g/180 mL/cup	0.003
instant tea	0.585	0.3 g/180 mL/cup	0.0002
cocoa	0.616	4 g/180 mL/cup	0.003
green tea	0.472	0.3 g/180 mL/cup	0.0001

[a] Not applicable. [b] One gram of material is dissolved in 180 mL (1 cup) of water.

major drawback of this method is that the derivative must be prepared under strongly acidic conditions which may cause undesirable reactions such as decomposition of carbohydrates, proteins, or lipids in samples. All aldehydes react readily with cysteamine under moderate conditions at pH 7 and room temperature to form thiazolidine. Volatile aldehydes in food samples have been satisfactorily analyzed using these thiazolidine derivatives (Hayashi and Shibamoto, 1985; Hayashi et al., 1986; Yasuhara and Shibamoto, 1989a,b).

The optimum derivatization conditions were obtained at pH 8 and 1-h reaction time (Yasuhara and Shibamoto, 1991). The recovery efficiency of 2-methylthiazolidine from an aqueous solution was 99.2 ± 0.3% (n = 3). The NPD detection limit of 2-methylthiazolidine was 16.7 pg that is equivalent to 7.1 pg of acetaldehyde (Yasuhara and Shibamoto, 1991).

Table I shows the results of acetaldehyde analysis in foods and beverages and the calculated amount of acetaldehyde intake for each food or beverage when consumed. The foods consumed without additional water are listed in group I and the foods consumed with the addition of a certain amount of water are listed in group II. The values are the mean of three replications, and a correction has been made for a blank value. Alcoholic beverages contain a large quantity of acetaldehyde because it forms during alcohol fermentation. It also forms readily from ethanol oxidation. One of the whiskey samples contained the greatest amount of acetaldehyde (101.90 ppm) among the samples used for this study. Beer contained the least amount of acetaldehyde (5–12 ppm) among the alcoholic beverages examined. The acetaldehyde content in alcoholic beverages tends to be roughly equivalent to the ethanol content. However, considerable differences in the acetaldehyde content of the same substance was observed. For example, sake A contained over 60 ppm of acetaldehyde while sake B contained only 15 ppm. The acetaldehyde content of whiskey A was almost 4 times of that of whiskey B. Adachi et al. (1991) reported that the acetaldehyde concentration was highest in a liqueur (55 ppm) and lowest in a shochu (5.5 ppm). They also found that sake and wine contained acetaldehyde in the levels of 30 and 31 ppm, respectively. Those results are roughly consistent with the present study.

Acetaldehyde contents in fruit juices were somewhat low: 12 ppm in apple juice and 10 ppm in orange juice. This finding was surprising because acetaldehyde contributes to fruit flavors, particularly in citrus juice (Umano and Shibamoto, 1988). Nelson and Hoff (1969) found 0.21–0.92 and 0.06–1.09 ppm levels acetaldehyde in three varieties of unprocessed tomatoes and processed tomato juice, respectively. They noted the qualitative and quantitative differences in volatile components, including acetaldehyde, among the raw and heat-processed samples.

Milk samples did not contain acetaldehyde in large quantities. It was not surprising that whole milk contained more acetaldehyde than nonfat milk did since fat is reportedly one of the major sources of carbonyl compounds, including acetaldehyde (Yasuhara and Shibamoto, 1991). However, there was no appreciable difference in the acetaldehyde content of regular and nonfat yogurt.

The aldehyde contents of nonalcoholic beverages such as coffee and tea were considerably lower than those of alcoholic beverages.

Acetaldehyde is generally recognized as safe by the FDA (1988). Even though inhalation studies using experimental animals have shown that acetaldehyde is capable of inducing nasal carcinomas (Feron et al., 1982), no data are available on the chronic oral toxicity of acetaldehyde. Information on the chronic oral toxicity of acetaldehyde including carcinogenecity is needed because it occurs in a large number of foods and beverages (Feron et al., 1991), particularly in alcoholic beverages (Table I). However, determination of the health risk associated with acetaldehyde found in foods and beverages was not within the scope of this study.

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