Gas Chromatographic/Mass Spectrometric Method for Analysis of Trace Carbonyl Compounds in Foods and Beverages

Akio Yasuhara,[†] Kuniaki Kawada,[‡] and Takayuki Shibamoto*[,§]

National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba Ibaraki 305, Japan, Niigata Prefectural Research Laboratory for Health and Environment, Sowa, Niigata 950-21, Japan, and Department of Environmental Toxicology, University of California, Davis, California 95616

A new analytical method to determine trace carbonyl compounds in foods and beverages by derivatization and gas chromatography/mass spectrometry has been developed. A total of 72 carbonyl compounds (including 10 aliphatic aldehydes, 5 monoterpene aldehydes and ketones, 10 aromatic aldehydes, 3 heterocyclic aldehydes, 10 halogenaged aromatic aldehydes and a ketone, 15 aliphatic ketones, 7 olefinic ketones, 3 aromatic ketones, 5 saturated cyclic ketones, 2 unsaturated cyclic ketones, and 2 heterocyclic ketones) were derivatized to corresponding thiazolidines with cysteamine. Mass spectra of the thiazolidine derivatives are reported. Furfural concentrations in the Japanese commercial alcohol beverage sake were determined according to this method. The concentrations ranged rrom 68.0 to 933 ng/mL and the median value was 280 ng/mL among 7 commercial sake brands.

Keywords: *Carbonyl compounds; furfural; mass spectra; thiazolidines*

INTRODUCTION

Carbonyl compounds, particularly volatile aldehydes, have received much attention, not only from flavor chemists but also from toxicologists. Volatile aldehydes such as acetaldehyde formed from oxidation or decomposition of lipids play both positive and negative roles in foods. For example, certain aldehydes contribute off-flavors to many foods and beverages (Harayama et al., 1991); however, they generate pleasant flavors in appropriate concentrations. Some volatile aldehydes such as formaldehyde are reportedly toxic (Albert et al., 1982; Feron et al., 1982).

Therefore, many methods for the analysis of carbonyl compounds, including highly volatile formaldehyde and less volatile fatty aldehydes, have been reported. Most methods involve derivatization with 2,4-dinitrophenylhydrazine (2,4-DNP) (van Hoof et al., 1985; Smith et al., 1989), *N*-benzylethanolamine (Kennedy and Hill, 1982) or *O*-(2,3,4,5,6-pentafluorobenzyl)oxyamine (Kobayashi et al., 1980; Le Lacheur et al., 1993). However, these methods require strong acidic and high-temperature conditions to obtain derivatives, which may alter the carbonyl compounds of interest or produce additional carbonyl compounds. Moreover, these derivatives produce syn and anti forms from aldehydes except formaldehyde. Multideterminationof a mixture of carbonyl compounds becomes extremely complex if two forms of each carbonyl compound are produced. Therefore, it is difficult to determine different carbonyl compounds simultaneously by using these derivatization methods.

Recently, we have reported analysis of volatile carbonyl compounds in foods (Yasuhara and Shibamoto, 1989a, 1991), in beverages (Miyake and Shibamoto, 1993), in cigarette smoke (Miyake and Shibamoto, 1995), and in automobile exhaust (Yasuhara and Shibamoto, 1994) using cysteamine derivatives. Cysteamine reacts with almost all carbonyl compounds under mild conditions (at room temperature and neutral pH), and the resulting derivatives, thiazolidines, contain a nitrogen atom that can be detected at an extremely low level by nitrogen−phosphorus detection (NPD). For example, detectabilities of formaldehyde, hexanal, and nonanal as a corresponding thiazolidine derivative are 5.8, 24.7, and 36.2 pg, respectively (Yasuhara and Shibamoto, 1989b). Trace levels of many volatile carbonyl compounds in the headspace of food samples have been satisfactorily analyzed using this method (Yasuhara and Shibamoto, 1991, 1995).

In the present study, nearly 72 standard thiazolidine derivatives were synthesized and their mass spectra were reported as an aid to trace carbonyl compound analysis in foods and beverages. Analysis of trace furfural in commercial sake brands (a Japanese alcoholic beverage) was conducted to validate the method.

EXPERIMENTAL PROCEDURES

Materials. Authentic carbonyl compounds were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Aldrich Chemical Co. (Milwaukee, WI). Cysteamine was obtained from Tokyo Kasei Industries (Tokyo, Japan). Ethyl acetate, sodium chloride, sodium bicarbonate, and anhydrous sodium sulfate were purchased from Wako Pure Chemical Industries. All of the water used was distilled water through a Milli-Q System (Millipore, Bedford, MA).

A standard stock solution of furfural (10 mg/mL) was prepared by adding 1 g of furfural to 100 mL of distilled water. Furfural was further purified by distillation under reduced pressure prior to use. Several concentrations of furfural for the calibration curve were obtained by diluting the standard

* Author to whom correspondence should be addressed [telephone (530) 752-4523; fax (530) 752-3394; e-mail tshibamoto@ucdavis.edu].

[†] National Institute for Environmental Studies.

[‡] Niigata Prefectural Research Laboratory for Health and Environment.

[§] University of California.

S0021-8561(98)00109-5 CCC: $15.00
Published on Web 06/26/1998

stock solution with an appropriate volume of distilled water. Seven commercial brands of sake were bought from a local market.

Preparation of Thiazolidine Derivatives from Carbonyl Compounds. A total 72 carbonyl compounds—including 10 aliphatic aldehydes, 5 monoterpene aldehydes and ketones, 10 aromatic aldehydes, 3 heterocyclic aldehydes, 10 halogenated aromatic aldehydes, 15 aliphatic ketones, 7 olefinic ketones, 3 aromatic ketones, 5 saturated cyclic ketones, 2 unsaturated cyclic ketones, and 2 heterocyclic ketones—were derivatized to corresponding thiazolidines with cysteamine. Each carbonyl compound (100 mg) was dissolved in a 5 mL methanol/water (1:1, v/v) solution. Cysteamine hydrochloride (0.75 g) was added to each solution and the pH of the solution was immediately adjusted to 8 with 6 N NaOH. After the solutions were stirred, they were allowed to stand for 3 h (all carbonyl compounds except aromatic compounds) or 24 h (aromatic compounds) at room temperature. After reaction, 10 mL of distilled water was added to each solution and extracted with 7 mL of ethyl acetate. After the extracts were dried over anhydrous sodium sulfate for 6 h, 0.1 μL of each extract was analyzed for the thiazolidine derivative by gas chromatograpy/mass spectrometry (GC/MS).

GC/MS. A Hewlett-Packard (HP) model 5972 mass spectrometer interfaced to an HP model 5890 series II gas chromatograph was used for measurement of mass spectra. The GC was equipped with a 50 m $\times$ 0.2 mm i.d. ($d_f = 0.2\ \mu$m) DB-5 fused silica capillary column (J&W Scientific, Folsom, CA). The oven temperature was held at 60 °C for 3 min and then programmed to 300 °C at 10 °C/min. The injector temperature was 250 °C, and the detector temperature was 300 °C. A linear velocity of helium carrier gas was 28.1 cm/s. The injection was made in a splitless mode, and the purge-off period was 1 min. The MS conditions were as follows: ion source pressure, 5×10^{-5} Torr; ion source temperature, 170 °C; and ionization energy, 75 eV.

Tests of pH Effect on Yield of 2-(2-Furyl)thiazolidine from Furfural and Cysteamine. An aqueous solution (200 mL) of furfural (1 mg/L), the pH value of which was adjusted to 5, 6, 7, 8, 9, or 10 with a 6 N NaOH solution, was placed in a 300 mL flask. After cysteamine hydrochloride (2.5 g) was added to the above solutions, they were stirred for 1 h with a magnetic stirrer at room temperature. Sodium chloride (30 g) was added to the reaction solutions, which were subsequently extracted with ethyl acetate (20 mL) three times. The extracts were combined and concentrated to 2.0 mL using a rotary evaporator. Twenty microliters of an *N*-methylacetamide solution (10 mg/mL in benzene) was added to all samples as an internal standard prior to GC analysis. Caution! Benzene is a very toxic agent.

Tests of Reaction Time Effect on Yield of 2-(2-Furyl)-thiazolidine from Furfural and Cysteamine. An aqueous solution (200 mL) of furfural (1 mg/L), the pH of which was adjusted to 6 with a sodium bicarbonate solution, was mixed with 2.5 g of cysteamine hydrochloride. The mixture was stirred for 15, 30, 45, 60, or 90 min with a magnetic stirrer at room temperature. The rest of the procedure for each solution was the same as that for the pH dependency test.

Sample Preparations for Furfural Analysis in Sake Samples. Each sake sample (200 mL), the pH value of which was adjusted to 6 immediately before use with a sodium bicarbonate solution, was placed in a 300 mL flask. After cysteamine hydrochloride (2.5 g) was added to the sample, it was stirred for 1 h with a magnetic stirrer at room temperature. The rest of the procedure for each solution was the same as that for the pH dependency test.

Analysis of Furfural as 2-(2-Furyl)thiazolidine in Samples. An HP model 5890A gas chromatograph equipped with a 30 m $\times$ 0.25 mm i.d. ($d_f = 0.25\ \mu$m) DB-Wax fused silica capillary column (J&W Scientific) and an NPD was used for quantitative analysis of furfural as 2-(2-furyl)thiazolidine according to the method reported previously (Ettre, 1967). The oven temperature was held at 40 °C for 2 min and then programmed to 200 °C at 6 °C/min and held for 15 min. The GC peak areas were integrated with a System Instruments Model Chromatocorder 21 integrator. The injector and detector temperatures were 250 and 280 °C, respectively. A linear velocity of helium carrier gas was 33.4 cm/s at 40 °C. The injection was made in a splitless mode, and the purge-off period was 1 min. A GC calibration curve for 2-(2-furyl)thiazolidine was prepared using the reaction product from furfural and cysteamine. *N*-Methylacetamide was used as a GC internal standard.

RESULTS AND DISCUSSION

Mass Spectra of Thiazolidines. MS data of thiazolidines prepared from carbonyl compounds are shown in Table 1. MS data of 2-alkylthiazolidines derived from aliphatic aldehydes have similar MS fragmentation patterns except that of unsubstituted thiazolidine (from formaldehyde). The characteristic base peak appears at *m/z* 88, which is a thiazolidine ring ion $(C_3H_6NS)^+$.

Thiazolidine derivatives of citral and citronellal showed combination of fragments from a thiazolidine ring ($M^+ - SH$, *m/z* 178 and 190, respectively) and a monoterpene moiety (C_3H_5, *m/z* 41). MS data of citral and citronellal derivatives exhibited base peak at *m/z* 88, which is the same as that of aliphatic carbonyl compounds. It is difficult to rationalize the fragmentations of carvone, menthone, and α-ionone derivatives, but carvone's base peak at 108 may be formed from β-cleavage of the thiazolidine ring ($M^+ - C_4H_7NS$) (Budzikiewicz et al., 1967). Menthone and α-ionone derivatives have a base peak at *m/z* 128 $(C_5H_9NS)^+$, which seemed to be formed from a thiazolidine ring.

Thiazolidines from aromatic aldehydes show a predominant parent peak except for 2-phenylthiazolidine, which did not show a parent peak. All derivatives except 2-phenylthiazolidine showed a fragment of the thiazolidine ring (*m/z* 88). A predominant peak at $M^+ - SH$ was observed in all spectra except 2-phenylthiazolidine. Additionally, a fragment from $M^+ - SH - CN$ is present in all spectra of this group.

Thiazolidine derivatives from three heterocyclic compounds exhibited similar fragmentation patterns. Generally, $M^+ - SH$ fragmentation occurs and then a methylene molecule fragments off from a thiazolidine ring via rearrangement of a heteroring. This fragmentation indicates that the thiazolidine ring is less stable than either the furan or the pyridine ring.

Thiazolidine derivatives from halogenated aromatic aldehydes show characteristic isotope peaks at $M^+ + 2$ except for 2-(3,4-difluorophenyl)thiazolidine. Generally a $M^+ - Cl$ peak appears as a base peak in the MS data of chlorinated compounds.

Aliphatic ketones give 2,2-dialkylthiazolidines. The longer alkyl group on C-2 leaves more readily from a molecular ion than the shorter alkyl group on C-2 does. Therefore, 2-ketone and 3-ketone derivatives give base peaks at *m/z* 102 $(C_4H_8NS)^+$ and at *m/z* 116 $(C_5H_{10}NS)^+$, respectively. The alkyl group (>C2) of the 2-alkyl-2-methylthiazolidine ion tends to cleave at the bonding between carbons 1 and 2 and subsequently forms the 2,2-dimethylthiazolidine ion (*m/z* 117) via absorption of a hydrogen radical. Therefore, 2-alkyl-2-methylthiazolidine exhibits a fragmentation pattern similar to that of 2,2-dimethylthiazolidine. The characteristic peaks of 2-alkyl-2-methylthiazolidine appear at *m/z* 84 $[(CH_3)_2C{-}N{=}CH{-}CH_3]^+$, *m/z* 70 $[(CH_3)_2C{-}N\ CH_2]^+$, and *m/z* 58 $[(CH_3)_2C{-}NH_2]^+$. All olefinic ketones used were 2-ketone, and therefore derivatives were 2-methyl-2-alkenylthiazolidines. Mass spectra of these derivatives

Table 1. Mass Spectra of Thiazolidine Derivatives

parent carbonyl compd	thiazolidine deriv	MS, *m/z* (intensity)
	Aliphatic Aldehydes	
formaldehyde	unsubstituted	M^+ = 89 (100), 88 (31), 61 (14), 60 (10), 59 (23), 45 (21), 43 (96), 30 (25)
acetaldehyde	2-methyl-	M^+ = 103 (61), 88 (100), 61 (19), 59 (30), 57 (69), 56 (98), 45 (31), 44 (63), 42 (40)
propanal	2-ethyl-	M^+ = 117 (9), 88 (100), 70 (24), 61 (14), 58 (11), 56 (20), 45 (12), 42 (14), 41 (16)
isobutanal	2-isopropyl-	M^+ = 131 (6), 88 (100), 61 (10), 38 (12), 36 (33)
butanal	2-propyl-	M^+ = 131 (12), 88 (100), 70 (13), 61 (13), 56 (22), 42 (16), 41 (11), 38 (28), 36 (84)
isopentanal	2-isobutyl-	M^+ = 145 (12), 88 (100), 70 (8), 61 (10), 56 (23), 42 (14), 38 (23), 36 (72), 35 (13)
pentanal	2-butyl-	M^+ = 145 (12), 88 (100), 70 (7), 61 (9), 56 (21), 42 (8), 41 (9), 38 (13), 36 (40), 30 (9)
hexanal	2-pentyl-	M^+ = 159 (9), 88 (100), 70 (8), 61 (9), 56 (26), 42 (9), 41 (13), 38 (21), 36 (60), 30 (13)
heptanal	2-hexyl-	M^+ = 173 (5), 88 (100), 70 (6), 61 (7), 56 (22), 41 (9), 38 (9), 36 (27), 30 (12)
octanal	2-heptyl-	M^+ = 187 (0), 88 (100), 70 (8), 61 (11), 56 (35), 41 (22), 38 (16), 36 (52), 30 (32)
	Monoterpene Aldehydes and Ketones	
citral	2-(2,6-dimethyl-1,5-heptadienyl)-	M^+ = 211 (1), 178 (2), 143 (17), 110 (8), 96 (19), 94 (14), 88 (100), 82 (12), 81 (11), 80 (11), 79 (10), 77 (11), 69 (19), 67 (18), 53 (17), 41 (72), 39 (29)
citronellal	2-(2,6-dimethyl-5-heptenyl)-	M^+ = 213 (1), 190 (2), 166 (28), 136 (9), 131 (12), 130 (32), 128 (16), 88 (100), 84 (11), 70 (20), 69 (18), 61 (16), 56 (32), 55 (16), 44 (15), 41 (58), 39 (15)
carvone	6-methyl-9-isopropenyl-1,4-thiazaspiro[5,6]-dec-6-ene	M^+ = 209 (96), 194 (29), 176 (34), 168 (23), 162 (72), 148 (28), 134 (60), 120 (75), 108 (100), 107 (25), 93 (29), 91 (47), 81 (29), 77 (36), 67 (21), 65 (23), 61 (66), 53 (38), 45 (14), 41 (70), 39 (55)
menthone	6-isopropyl-9-methyl-1,4-thiazaspiro[5,6]decane	M^+ = 213 (35), 198 (83), 180 (19), 171 (31), 166 (20), 156 (33), 138 (23), 128 (100), 124 (57), 114 (16), 101 (13), 96 (14), 81 (10), 69 (17), 61 (39), 55 (26), 43 (22), 41 (62), 39 (22)
α-ionone	2-methyl-2-[2-(2,6,6-trimethyl-2-cyclohexen-yl)-1-ethenyl]-	M^+ = 251 (6), 236 (12), 128 (100), 120 (11), 102 (11), 91 (13), 42 (16), 41 (21), 39 (9)
	Aromatic Aldehydes	
benzaldehyde	2-phenyl-	M^+ = 165 (0), 109 (22), 77 (20), 44 (43), 43 (7), 42 (9), 36 (9), 30 (100)
o-tolualdehyde	2-(2-methyl-	M^+ = 179 (56), 178 (31), 146 (54), 135 (22), 132 (48), 120 (20), 119 (23), 118 (100), 117 (36), 105 (39), 88 (14), 77 (18), 45 (10)
m-tolualdehyde	2-(3-methylphenyl)-	M^+ = 179 (85), 178 (62), 146 (18), 132 (81), 120 (30), 119 (24), 118 (100), 117 (28), 88 (20), 77 (18), 65 (20), 45 (10)
p-tolualdehyde	2-(4-methylphenyl)-	M^+ = 179 (78), 178 (63), 146 (19), 132 (75), 120 (28), 119 (26), 118 (100), 117 (23), 105 (58), 91 (39), 89 (11), 88 (11), 77 (19), 65 (19), 45 (10)
cinnamaldehyde	2-(2-phenyl-1-ethenyl)-	M^+ = 191 (75), 190 (45), 158 (16), 144 (60), 130 (89), 115 (100), 103 (21), 91(25), 88 (18), 77 (25), 45 (12)
2,4-dimethylbenzaldehyde	2-(2,4-dimethylphenyl)-	M^+ = 193 (51), 192 (27), 160 (51), 146 (31), 133 (30), 132 (100), 119 (36), 117 (25), 91 (25), 88 (8), 77 (18), 45 (7)
2,4,5-trimethylbenzaldehyde	2-(2,4,5-trimethylphenyl)-	M^+ = 207 (52), 206 (23), 174 (60), 163 (20), 160 (24), 147 (38), 146 (100), 145 (17), 133 (26), 131 (22), 130 (18), 105 (16), 88 (9), 45 (7)
2,4,6-trimethylbenzaldehyde	2-(2,4,6-trimethylphenyl)-	M^+ = 207 (15), 206 (10), 174 (91), 160 (20), 147 (25), 146 (100), 145 (21), 144 (18), 131 (29), 130 (23), 91 (19)
p-*tert*-butylbenzaldehyde	2-(4-*tert*-butylphenyl)-	M^+ = 221 (81), 220 (68), 188 (28), 174 (24), 162 (29), 146 (40), 118 (100), 117 (23), 91 (35), 88 (18), 77 (11), 57 (36), 45 (7)
p-isobutylbenzaldehyde	2-(4-isobutylphenyl)-	M^+ = 221 (87), 220 (79), 188 (33), 174 (48), 164 (18), 162 (36), 119 (36), 118 (100), 117 (34), 91 (56), 88 (15), 77 (13), 57 (15)
	Heterocyclic Aldehydes	
furfural	2-(2-furyl)-	M^+ = 155 (100), 122 (9), 109 (37), 108 (25), 96 (35), 95 (24), 94 (29), 81 (32), 80 (20), 69 (9), 53 (10), 52 (10), 51 (9), 45 (10), 41 (10), 39 (21), 36 (8)
5-methylfurfural	2-(5-methylfuran-2-yl)-	M^+ = 169 (100), 154 (12), 136 (14), 123 (47), 122 (31), 109 (42), 108 (88), 95 (53), 94 (23), 81 (17), 80 (18), 79 (21), 53 (21), 45 (11), 39 (14)
6-methylpyridine	2-(6-methylpyridine-2-yl)-	M^+ = 180 (4), 148 (11), 147 (100), 133 (56), 121 (21), 119 (66), 106 (35), 93 (31), 88 (21), 65 (19)
	Halogenated Aromatic Aldehydes and a Ketone	
p-chloromethylbenzaldehyde	2-(4-chloromethylphenyl)-	M^+ = 213 (100), 215 (39), 212 (83), 178 (71), 168 (30), 166 (84), 154 (58), 139 (48), 132 (47), 118 (64), 117 (68), 91 (96), 90 (43), 89 (45), 88 (29), 77 (32), 45 (24)

Table 1. (Continued)

parent carbonyl compd	thiazolidine deriv	MS, *m*/*z* (intensity)
	Halogenated Aromatic Aldehydes and a Ketone	
2,3-dichlorobenzaldehyde	2-(2,3-dichlorophenyl)-	M^+ = 233 (13), 235 (9), 200 (43), 198 (100), 188 (38), 186 (56), 176 (17), 174 (36), 172 (21), 159 (37), 149 (12), 117 (13), 75 (15), 45 (12)
3,4-dichlorobenzaldehyde	2-(3,4-dichlorophenyl)-	M^+ = 233 (75), 235 (49), 234 (40), 232 (28), 200 (22), 188 (65), 186 (100), 176 (29), 174 (63), 172 (40), 161 (39), 159 (60), 149 (27), 123 (21), 88 (26), 45 (20)
2,4-dichlorobenzaldehyde	2-(2,4-dichlorophenyl)-	M^+ = 233 (35), 235(24), 200 (51), 198 (100), 189 (25), 188 (58), 186 (86), 174 (67), 172 (38), 161 (40), 159 (61), 152 (23), 123 (24), 117 (25), 88 (19), 45 (21)
2,3,5-trichlorobenzaldehyde	2-(2,3,5-trichlorophenyl)-	M^+ = 267 (9), 236 (19), 234 (76), 232 (100), 222 (36), 220 (38), 210 (26), 208 (39), 206 (19), 195 (34), 193 (36), 186 (20), 151 (24), 88 (29), 60, (16), 45 (15)
o-bromobenzaldehyde	2-(2-bromophenyl)-	M^+ = 243 (17), 245 (17), 198 (36), 196 (36), 186 (18), 184 (34), 171 (28), 169 (28), 164 (100), 117 (26), 89 (22), 88 (17), 77 (17), 45 (11)
m-bromobenzaldehyde	2-(3-bromophenyl)-	M^+ = 243 (99), 245 (100), 242 (77), 210 (24), 198 (96), 196 (97), 186 (47), 184 (91), 171 (64), 169 (67), 117 (61), 90 (36), 89 (43), 88 (66), 77 (34), 50 (29), 45 (25)
p-bromobenzaldehyde	2-(4-bromophenyl)-	M^+ = 243 (91), 245 (90), 242 (78), 99 (36), 198 (99), 196 (99), 186 (46), 184 (100), 182 (54), 171 (65), 169 (67), 117 (56), 89 (45), 88 (28), 77 (29), 50 (28), 45 (22)
3,4-difluorobenzaldehyde	2-(3,4-difluorophenyl)-	M^+ = 201 (60), 200 (37), 168 (10), 155 (18), 154 (100), 142 (24), 140 (31), 127 (58), 88 (10), 45 (10)
3,4-dichloroacetophenone	2-methyl-2-(3,4-dichlorophenyl)-	M^+ = 247 (52), 249 (36), 234 (41), 232 (58), 214 (26), 202 (65), 200 (100), 190 (61), 188 (93), 174 (39), 172 (61), 161 (51), 159 (78), 102 (31), 75 (20), 59 (22), 42 (52)
	Aliphatic Ketones	
acetone	2,2-dimethyl-	M^+ = 117 (72), 102 (54), 84 (13), 71 (51), 70 (99), 58 (100), 42 (83), 41 (30), 30 (43)
methyl ethyl ketone	2-methyl-2-ethyl-	M^+ = 131 (29), 102 (100), 84 (63), 72 (34), 61 (30), 56 (47), 42 (68), 41 (17), 30 (26)
3-methyl-2-butanone	2-methyl-2-isopropyl-	M^+ = 145 (5), 102 (100), 98 (20), 84 (13), 61 (26), 42 (19), 30 (9)
2-pentanone	2-methyl-2-propyl-	M^+ = 145 (29), 130 (25), 102 (100), 98 (34), 86 (24), 84 (25), 70 (35), 61 (30), 56 (28), 58 (13), 42 (55), 41 (30), 30 (22)
3-methyl-2-pentanone	2-methyl-2-(1-methylpropyl)-	M^+ = 159 (6), 144 (11), 102 (100), 98 (11), 84 (22), 61 (37), 56 (12), 55(12), 42 (35), 41 (15), 30 (8)
2-hexanone	2-methyl-2-butyl-	M^+ = 159 (23), 144 (15), 130 (34), 126 (15), 117 (31), 102 (100), 84 (27), 70 (61), 61 (31), 58 (32), 56 (26), 42 (56), 41 (29), 30 (23)
isoamyl methyl ketone	2-methyl-2-(3-methylbutyl)-	M^+ = 173 (8), 158 (11), 130 (51), 117 (53), 102 (100), 84 (22), 70 (78), 61 (29), 58 (41), 56 (23), 43 (20), 42 (63), 42 (43), 39 (22), 30 (26)
2-heptanone	2-methyl-2-pentyl-	M^+ = 173 (17), 158 (14), 130 (44), 126 (21), 117 (25), 102 (100), 84 (25), 70 (55), 61 (27), 58 (28), 56 (23), 42 (48), 41 (28), 30 (21)
2-octanone	2-methyl-2-hexyl-	M^+ = 187 (18), 172 (13), 130 (61), 117 (28), 102 (100), 84 (23), 70 (58), 61 (27), 58 (31), 56 (23), 42 (45), 41 (32), 30 (20)
2-nonanone	2-methyl-2-heptyl-	M^+ = 201 (15), 186 (14), 130 (55), 117 (21), 102 (100), 84 (22), 70 (50), 61 (25), 58 (21), 56 (26), 42 (58), 41 (51), 39 (21), 30 (15)
3-pentanone	2,2-diethyl-	M^+ = 145 (14), 117 (6), 116 (100), 98 (38), 70 (16), 61 (19), 56 (36), 41 (14), 30 (13)
3-hexanone	2-ethyl-2-propyl-	M^+ = 159 (20), 144 (7), 130 (92), 116 (100), 112 (34), 98 (19), 84 (21), 70 (39), 61 (30), 56 (36), 41 (34), 39 (23), 30 (14)
3-heptanone	2-ethyl-2-butyl-	M^+ = 173 (20), 145 (8), 144 (100), 127 (20), 116 (84), 102 (24), 98 (16), 84 (45), 61 (30), 56 (31), 30 (15)
4-heptanone	2,2-dipropyl-	M^+ = 173 (11), 158 (9), 140 (20), 130 (100), 102 (8), 61 (20), 41 (27), 39 (13), 30 (7)
3-hydroxy-3-methyl-2-butanone	2-methyl-2-(1-hydroxy-1-methylethyl)-	M^+ = 161 (0), 146 (4), 102 (100), 96 (9), 86 (10), 61 (28), 59 (22), 43 (18), 42 (39)
	Olefinic Ketones	
5-methyl-3-hexen-2-one	2-methyl-2-(3-methyl-1-butenyl)-	M^+ = 171 (8), 102 (100), 82 (9), 61 (13), 41 (28), 41 (20), 39 (12)
2-methyl-2-hepten-6-one	2-methyl-2-(4-methyl-3-pentenyl)-	M^+ = 185 (24), 170 (12), 117 (33), 110 (20), 102 (100), 84 (11), 70 (55), 69 (27), 61 (25), 58 (34), 42 (59), 41 (76), 39 (31), 30 (20)
mesityl oxide	2-methyl-2-(2-methyl-1-propenyl)-	M^+ = 157 (92), 142 (11), 129 (42), 114 (58), 96 (29), 60 (70), 59 (44), 55 (100), 54 (23), 45 (30), 42 (43), 41 (56), 39 (53)
3-hepten-2-one	2-methyl-2-(1-pentenyl)-	M^+ = 171 (6), 156 (4), 102 (100), 96 (8), 61 (14), 59 (10), 42 (28), 41 (17), 39 (12)
3-octen-2-one	2-methyl-2-(1-hexenyl)-	M^+ = 185 (7), 170 (3), 110 (8), 102 (100), 96 (9), 59 (9), 42 (25), 41 (14), 39 (10)
3-nonen-2-one	2-methyl-2-(1-heptenyl)-	M^+ = 199 (58), 184 (8), 156 (23), 128 (37), 102 (38), 94 (44), 82 (84), 60 (98), 55 (100), 45 (35), 42 (57), 41 (85), 39 (43)
4-acetyl-1-methyl-1-cyclohexene	2-methyl-2-(4-methyl-3-cyclohexenyl)-	M^+ = 197 (42), 182 (16), 164 (17), 122 (16), 108 (7), 102 (100), 61 (21), 42 (30), 39 (13)

Table 1. (Continued)

parent carbonyl compd	thiazolidine deriv	MS, *m/z* (intensity)
	Aromatic Ketones	
acetophenone	2-methyl-2-phenyl-	M^+ = 179 (55), 164 (60), 132 (100), 120 (56), 104 (58), 91 (75), 77 (49), 42 (35)
p-ethylacetophenone	2-methyl-2-(4-methylphenyl)-	M^+ = 207 (64), 192 (63), 174 (38), 160 (88), 148 (55), 147 (25), 132 (100), 131 (38), 119 (74), 117 (28), 91 (25), 77 (21), 42 (23), 39 (9)
cyclohexyl phenyl ketone	2-cyclohexyl-2-phenyl-	M^+ = 247 (7), 200 (20), 164 (100), 104 (33), 91 (10), 77 (77), 41 (9), 39 (7)
	Saturated Cyclic Ketones	
cyclopentanone	1,4-thiazaspiro[5,5]nonane	M^+ = 143 (44), 114 (100), 96 (75), 84 (18), 67 (16), 61 (16), 54 (28), 41 (24), 39 (18), 30 (10)
cyclohexanone	1,4-thiazaspiro[5,6]decane	M^+ = 157 (67), 114 (93), 110 (100), 98 (20), 82 (23), 61 (20), 54 (28), 41 (36), 39 (21), 30 (15)
4-ethylcyclohexanone	8-ethyl-1,4-thiazaspiro-[5,6]decane	M^+ = 185 (34), 156 (28), 114 (100), 101 (19), 96 (19), 82 (23), 61 (15), 54 (18), 41 (25), 39 (11), 30 (9)
2-propylcyclohexanone	6-propyl-1,4-thiazaspiro-[5,6]decane	M^+ = 199 (34), 170 (40), 157 (30), 156 (31), 152 (23), 114 (100), 110 (42), 101 (22), 67 (11), 61 (17), 41 (37), 39 (16), 30 (6)
2-hydroxy-3-methyl-2-cyclopentenone	6-hydroxyl-7-methyl-1,4-thiazaspiro[5,5]non-6-ene	M^+ = 171 (18), 156 (2), 124 (100), 101 (20), 96 (21), 68 (32), 67 (26), 61 (21), 55 (28), 54 (50), 53 (25), 41 (36), 39 (34), 30 (6)
	Unsaturated Cyclic Ketones	
2-pentyl-2-cyclopenten-1-one	6-pentyl-1,4-thiazaspiro-[5,5]non-6-ene	M^+ = 211 (33), 182 (72), 178 (29), 168 (27), 164 (62), 154 (33), 122 (100), 120 (27), 108 (51), 94 (40), 79 (35), 77 (33), 67 (20), 65 (20), 61 (33), 41 (37), 39 (23)
2-cyclohexen-1-one	1,4-thiazaspiro[5,6]dec-6-ene	M^+ = 155 (45), 109 (22), 108 (100), 94 (32), 93 (20), 80 (20), 79 (41), 77 (46), 67 (35), 61 (25), 58 (25), 51 (25), 46 (24), 45 (30), 41 (48), 39 (50), 30 (18)
	Heterocyclic Ketones	
3-acetyl-2,5-dimethylfuran	2-methyl-2-(2,5-dimeth-ylfuran-3-yl)-	M^+ = 197 (75), 182 (32), 164 (69), 150 (31), 137 (31), 122 (50), 121 (43), 120 (65), 109 (28), 108 (78), 94 (18), 77 (30), 51 (21), 43 (100), 42 (34)
2-methyltetrahydro-furan-3-one	1-methyl-2,6,9-oxathiaza-spiro[5,5]nonane	M^+ = 159 (23), 115 (84), 114 (100), 88 (5), 61 (21), 60 (28), 59 (26), 54 (21), 43 (22), 39 (9), 30 (6)

show complex fragmentation patterns, but a predominant peak appeared at *m/z* 102 $(C_4H_8NS)^+$.

In contrast to 2-alkyl-2-methylthiazolidines, 2-methyl-2-phenylthiazolidines derived from methyl phenyl ketones cleaved a 2-methyl substituent off over a 2-phenyl substituent from a thiazolidine ring and gave a large peak at $M^+ - CH_3$. In the case of 2-cyclohexyl-2-phenylthiazolidine, the cyclohexyl group cleaved off from a molecular ion and gave a base peak at *m/z* 164.

Cyclic ketones produced spirocyclic thiazolidines. Thiazolidine derivatives from saturated cyclic ketones gave a predominant fragment ion at *m/z* 114 $(C_5H_8NS)^+$, which may form from a 2,2-dimethylthiazole ion. Thiazolidines from unsaturated cyclic ketones possessed a characteristic fragment at *m/z* 108. A derivative with a substituent at the carbon 2 atom on a spiro ring did not have a fragment at *m/z* 108, suggesting that this fragment at *m/z* 108 is $(2\text{-ethenylthazolidine} - H)^+$.

Thiazolidine derivatives of heterocyclic ketones showed complex fragmentation patterns. The 3-acetyl-2,5-dimethylfuran derivative showed an acetyl ion $(CH3CO)^+$ (*m/z* 43) as a base peak. On the other hand, the 2-methyltetrahydrofuran-3-one derivative possessed a characteristic ion for spiro thiazolidine at *m/z* 114 $(C_5H_8NS)^+$.

Investigation of MS fragmentation of thiazolidine derivatives from carbonyl compounds is not a major objective of this study. However, we hope that mass spectra of thiazolidine derivatives reported here will help to detect trace levels of carbonyl compounds in foods and beverages.

Analysis of Furfural in Commercial Sake Brands. Analysis of furfural in a typical Japanese alcoholic beverage, sake, was performed using a standard 2-furylthaizolidine synthesized in this experiment. Furfural is known to form from sugar degradation and possesses a sweet caramel-like flavor (Hodge, 1967). Also, it has been reported to be present in many foods and beverages, including alcoholic spirits (van Straten et al., 1978; Lo Coco et al., 1992), bread, apple juice, baked sesame, coffee, tea (Shiraki, 1969; Yamanishi, 1981), and heated milk (Kanizawa, 1980). Furfural has often been detected in heat-processed or fermented foods; for example, it is found in bread but not in wheat flour and in soy sauce and soy paste but not in soybeans (Yoshizawa, 1980).

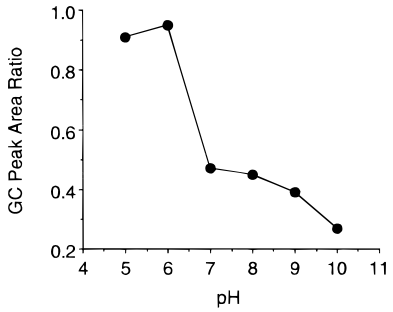

Figure 1. Effect of pH on the formation of 2-(2-furyl)-thiazolidine from furfural and cysteamine. Peak area ratio equals peak area of 2-(2-furyl)thiazolidine/internal standard (*N*-methylacetamide).

The optimum pH for the reaction between furfural and cysteamine ranged from 5.0 to 6.0 (Figure 1), which is not consistent with aliphatic saturated aldehydes, which require weak basic conditions for derivatization. A weakly acidic condition is favorable both for furfural and for 2-furylthiazolidine, because polymerization or oxidation occurrs readily under alkaline or strong acidic conditions. Therefore, the other methods, such as the

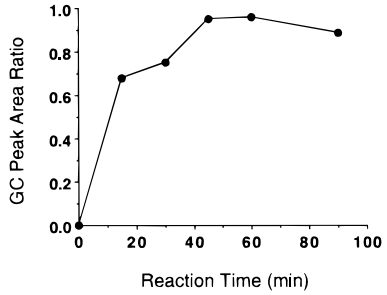

Figure 2. Effect of reaction time on the formation of 2-(2-furyl)thiazolidine from furfural and cysteamine. Peak area ratio equals peak area of 2-(2-furyl)thiazolidine/internal standard (*N*-methylacetamide).

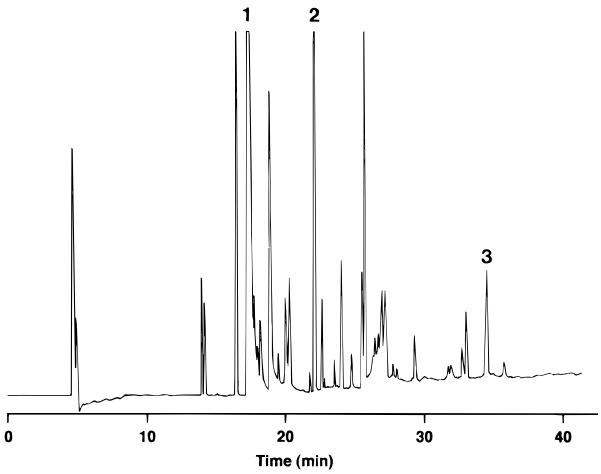

Figure 3. Typical gas chromatogram of the extract from the sake sample (brand IV): 1, unreacted cysteamine; 2, internal standard (*N*-methylacetamide), 3, 2-(2-furyl)thiazolidine (furfural derivative).

use of the 2,4-dinitrophenylhydrozine derivative, which required a strong acidic condition (pH 2), could not be used to compare the results from the present method. The reaction between furfural and cysteamine appeared to be complete after 45 min (Figure 2). However, reaction mixtures were stirred for an additional 15 min.

Recovery efficiency of furfural was investigated by five replicate 200 mL of brand IV sake sample (Table 1) spiked with furfural (1 μg/mL) under the conditions used for the above commercial sake samples. Recovery efficiency was 91.2 $\pm$ 19.1%. The value is mean $\pm$ standard deviation ($n = 5$).

Although the results showed significant deviation on each measurement, quantification was effectively performed by using the ratio of peak areas of product against an internal standard, *N*-methylacetamide (Ettre, 1967).

The lowest detection limit (DL) of furfural as 2-(2-furyl)thiazolidine was 195 μg/L, with an NPD in the present study. No peaks in blank tests were observed at the position corresponding to 2-(2-furyl)thiazolidine.

Figure 3 shows a typical gas chromatogram of an extract from a sake sample. The baseline resolution of 2-(2-furyl)thiazolidine (furfural derivative) was obtained. Table 2 shows the analytical results of furfural in commercial sake samples. The concentration ranged from 68.0 to 933 ng/mL, and the median value was 280 ng/mL. Sakes III and VII, which were stored in a refrigerator at 5 °C, had lower concentrations of furfural than did other sakes (except I), suggesting that higher temperatures promote the formation of furfural. Concentrations reported in the literature (Lo Coco et al., 1992) for alcoholic beverages were 1.15−6.05 μg/mL for cognacs, 42.2−269 ng/mL for brandies, 85.4−106 ng/mL for vodkas, and 5.38−7.58 μg/mL for whiskeys. The concentrations of furfural in sakes were similar to those in vodkas.

Table 2. Results of Furfural Analysis in Commercial Sake Brands

sake brand	concn (ng/mL)	sake brand	concn (ng/mL)
I	148	V	933
II	435	VI	280
III	198	VII	68.0
IV	535		

Furfural plays an important role in the flavors of alcoholic beverages because in proper dilution it possesses a sweet, caramel-like taste (Arctander, 1969). In particular, this is true in sake flavor because sake is generally heated before drinking and the heat probably produces additional furfural. There have been no reports on analysis of furfural in sake prior to the present study.

LITERATURE CITED

Albert, R. E.; Sellakumar, A. R.; Laskin, S.; Juschner, M.; Nelson, N.; Snyder, C. A. Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. *J. Natl. Cancer Inst.* **1982**, *68*, 597−603.

Arctander, S. *Perfume and Flavor Chemicals*; published by the author: Montclair, NJ, 1969; no. 1413.

Budzikiewicz, H.; Djerassi, C.; Williams, D. H. In *Mass Spectrometry of Organic Compounds*; Holden-Day: San Francisco, 1967; pp 284−286.

Ettre, L. S. Interpretation of analytical results. In *The Practice of Gas Chromatography*; Ettre, L. S., Zlatkis, A., Eds.; Interscience Publishers: New York, 1967; p 402.

Feron, V. J.; Kruysse, A.; Woutersen, R. A. Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo[*a*]pyrene or diethylnitrosamine. *Eur. J. Cancer Clin. Oncol.* **1982**, *18*, 13−31.

Harayama, K.; Hayase, F.; Kato, H. Evaluation by multivariate analysis of off-flavor in headspace volatiles formed during storage of beer. *Agric. Biol. Chem.* **1991**, *55*, 393−398.

Hodge, J. Origin of flavor in foods nonenzymatic browning reactions. In *Chemistry and Physiology of Flavors*; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; AVI Publishing: Westport, CT, 1967; pp 465−491.

Kanizawa, T. In *Dictionary for Flavor*; Fujimaki, M., Hattori, T., Hayashi, K., Arai, S., Eds.; Asakura Shoten: Tokyo, 1980; pp 191−198.

Kennedy, E. R.; Hill, Jr., R. H. Determination of formaldehyde in air as an oxazolidine derivative by capillary gas chromatography. *Anal. Chem.* **1982**, *54*, 1739−1742.

Kobayashi, K.; Tanaka, M.; Kawai, S. Gas chromatographic determination oflow-molecular-weight carbonyl compounds in aqueous solution as their O-(2,3,4,5,6-pentafluorobenzyl)-oximes. *J. Chromatogr.* **1980**, *187*, 413−417.

Le Lacheur, R. M.; Sonnenberg, L. B.; Singer, P. C.; Christman, R. F.; Charles, M. J. Identification of carbonyl compounds in environmental samples. *Environ. Sci. Technol.* **1993**, *27*, 2745−2753.

Lo Coco, F.; Ceccon, L.; Valentini, C.; Novelli, V. High-performance liquid chromatographic determination of 2-furaldehyde in spirits. *J. Chromatogr.* **1992**, *590*, 235−240.

Miyake, T.; Shibamoto, T. Quantitative analysis of acetaldehyde in foods and beverages. *J. Agric. Food Chem.* **1993**, *41*, 1968−1970.

Miyake, T.; Shibamoto, T. Quantitative analysis by gas chromatography of volatile carbonyl compounds in cigarette smoke. *J. Chromatogr. A* **1995**, *693*, 376−381.

Shiraki, Z. In *Flavor of Foods*; Korin Shoin: Tokyo, 1969; pp 178−227.

Smith, D. F.; Kleindienst, T. E.; Hudgens, E. E. Improved high-performance liquid chromatographic method for artifact-free measurements of aldehydes in the presence of ozone using 2,4-dinitrophenylhydrazine. J. *Chromatogr.* **1989**, *483*, 431−436.

van Hoof, F.; Wittocx, A.; van Buggenhout, E.; Janssens, J. Determination of aliphatic aldehydes in waters by high-performance liquid chromatography. *Anal. Chim. Acta* **1985**, *169*, 419−424..

van Straten, S.; Jonk, G.; van Gemert, L. Alteration in a wine distillate during aging. In *Flavor of Foods and Beverages*; Charalambous, G., Inglett, G. E., Eds.; Academic Press: New York, 1978; pp 381−390.

Yamanishi, T. The aroma of various teas. In *Flavor of Foods and Beverages*; Charalambous, G., Inglett, G. E., Eds.; Academic Press: New York, 1978; pp 305−337.

Yasuhara, A.; Shibamoto, T. Analysis of aldehydes and ketones in the headspace of heated pork fat. *J. Food Sci*. **1989a**, *54*, 1471−1472, 1484.

Yasuhara, A.; Shibamoto, T. Determination of physical and spectral data on thiazolidines for trace aldehyde analysis. *Agric. Biol. Chem*. **1989b**, *53*, 2273−2274.

Yasuhara, A.; Shibamoto, T. Determination of volatile aliphatic aldehydes in the headspace of heated food oils by derivatization with 2-aminoethanethiol. *J. Chromatogr.* **1991**, *547*, 291−298.

Yasuhara, A.; Shibamoto, T. Gas chromatographic determinatin of trace aldehydes in automobile exhaust exhaust by a cysteamine derivatization method. *J. Chromatogr.* **1994**, *672*, 261−266.

Yasuhara, A.; Shibamoto, T. Quantitative analysis of volatile aldehydes formed from various kinds of fish flesh during heat treatment. *J. Agric. Food Chem*. **1995**, *43*, 94−97.

Yoshizawa, S. The flavor of fermented foods. In *Dictionary for Flavor*; Fujimaki, M., Hattori, T., Hayashi, K., Arai, S., Eds.; Asakura Shoten: Tokyo, 1980; pp 198−228.

Received for review February 6, 1998. Revised manuscript received May 21, 1998. Accepted May 27, 1998.

JF980109E