Analysis of Methyl Glyoxal in Foods and Beverages

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Trace quantities of the mutagen methyl glyoxal in foods and beverages were determined by a newly developed method. Methyl glyoxal was reacted with cysteamine to give 2-acetylthiazolidine in a food or a beverage sample at pH 6. 2-Acetylthiazolidine formed from methyl glyoxal was extracted with dichloromethane and subsequently analyzed by a gas chromatograph equipped with a fused silica capillary column and a thermionic detector. A total of 17 commercial food items were analyzed for methyl glyoxal. The quantities of methyl glyoxal in foods ranged from 0.04 (orange juice) to 47 ppm (decaffeinated brewed coffee).

Methyl glyoxal has been found in many foods such as bread, boiled potatoes, caramelized sucrose, and roasted coffee (Wiseblatt and Kohn, 1960; Lukesch, 1956; Kajita and Senda, 1972; Kasai et al., 1982). Methyl glyoxal is also known as a sugar fragmentation product and is one of the most highly reactive compouds in a browning rection (Hodge, 1953). Methyl glyoxal was strongly mutagenic toward *Salmonella typhimurium* TA100 without metabolic activation and was moderately mutagenic toward *Escherichia coli* WP2 *uvr*A and *Escherichia coli* WP2 *uvr*A pKM101. Kasai et al. (1982) estimated that methyl glyoxal contributes 50% of the total mutagenicity of coffee (Kasai et al., 1982).

In spite of increasing concern about the levels of methyl glyoxal in foods and food products, there are virtually no reports on analytical methods for methyl glyoxal. It has been determined as 2-methylquinoxaline by gas chromatography after reaction with *o*-phenylenediamine (Moree-Testa and Saint-Jalm, 1981). Recently, methyl glyoxal was analyzed in coffee by this method (Kasai et al., 1982). The recovery efficiency or reactivity of methyl glyoxal toward *o*-phenylenediamine, however, has not yet been thoroughly studied.

The approach of the present study was suggested by an earlier study of one of the authors in which Maillard browning reaction model systems consisting of aldehydes and cysteamine produced many thiazolidine derivatives including 2-acetylthiazolidine by derivation from methyl glyoxal (Sakaguchi and Shibamoto, 1978). This earlier study indicated that aldehydes such as methyl glyoxal can be detected as thiazolidine derivatives in an aqueous sample. 2-Acetylthiazolidine is much more stable than methyl glyoxal and is sufficiently volatile for gas chromatography. Because 2-acetylthiazolidine contains a nitrogen atom, the highly sensitive and selective thermionic specific detector may be used for analysis. The purpose of this study then was to determine whether use of this thiazolidine derivative for methyl glyoxal analysis gives satisfactory results and, if so, to determine the levels of methyl glyoxal in commercial foods and beverages.

EXPERIMENTAL SECTION

Materials. Cysteamine hydrochloride, methyl glyoxal (40% in water), and *N*-methylacetamide were purchased from Aldrich Chemical Co., Milwaukee, WI. The extraction solvent dichloromethane was obtained from J. T. Baker, Chemical Co., Phillipsburg. NJ. The standard stock solution of *N*-methylacetamide was prepared by adding

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Table I. Proportions (%) of Each of the Three Reaction Products of Methyl Glyoxal and Cysteamine at Three Different Reaction Temperatures

temp, °C	2-acetyl-thiazoline	2-acetyl-thiazolidine	2-formyl-2-methylthiazolidine
0	2.0	40.0	58.0
25	8.5	89.0	2.5
100	81.0	19.0	0

10 mg of *N*-methylacetamide to 1 mL of solvent and was stored at 5 °C.

Instrumental Analysis. A Hewlett-Packard Model 5880A gas chromatograph (GC) equipped with a HP thermionic detector and a 50 m × 0.25 mm i.d. fused silica capillary column coated with Carbowax 20M was used for quantitative analysis of 2-acetylthiazolidine derived from methyl glyoxal with cysteamine. The oven temperature was programmed from 70 to 180 °C at 2 °C/min, and peak areas were integrated with a HP 5880A series GC terminal. The injector and detector temperatures were 250 °C. The hydrogen, air, and makeup gas (nitrogen) flow rates for a thermionic detector were 3, 60, and 20 mL/min, respectively.

Reaction of Cysteamine and Methyl Glyoxal. Cysteamine hydrochloride (0.75 g) was added to 250 mL of deionized water that contained 0.57 mg of methyl glyoxal. The solid cysteamine hydrochloride was added directly to the methyl glyoxal solution because the cysteamine hydrochloride solution was not stable enough to store as a standard stock solution for more than 3 days. The aqueous solution of cysteamine hydrochloride has pH 4. The solution was stirred with a magnetic stirrer for 30 min at room temperature. The reaction mixture was extracted with 70 mL of dichloromethane by a liquid–liquid continuous extractor for 6 h. The extract was dried over anhydrous sodium sulfate for 12 h. After removal of sodium sulfate, 10 μL of *N*-methylacetamide standard solution was added to the extract as a GC internal standard. The same reactions were conducted at pH 5–8. The pHs of the reaction solutions were adjusted with 6 N NaOH solution immediately after cysteamine hydrochloride was added. Otherwise, the reactions were conducted the same way as the first reaction at pH 4. Effect of pH on 2-acetylthiazolidine is shown in Figure 1. 2-Acetylthiazolidine recovery was highest at pH 6.

The gas chromatogram of the extract indicated that methyl glyoxal and cysteamine produced three products: 2-acetylthiazoline, 2-acetylthiazolidine, 2-formyl-2-methylthiazolidine. It would be ideal if 2-acetylthiazolidine were formed exclusively. The optimum reaction condition for 2-acetylthiazolidine was, therefore, determined by the following experiments:

(*1*) *Reaction Temperature.* The same molar ratio (0.1/0.1) of methyl glyoxal and cysteamine hydrochloride

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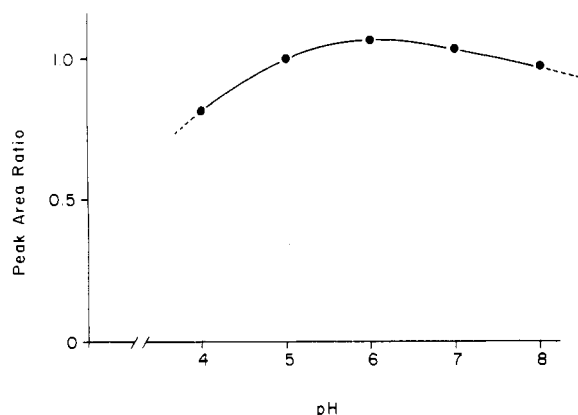*J. Agric. Food Chem.*, Vol. 33, No. 6, 1985 **1091**

Figure 1. Effect of pH on the formation of 2-acetylthiazolidine from methyl glyoxal. Peak area ratio equals peak area of 2-acetylthiazolidine/standard (*N*-methylacetamide).

Table II. Recovery Efficiency of Methyl Glyoxal as Acetylthiazolidine from an Aqueous Solution in the Presence of D-Glucose

concn D-glucose, %	yield, %
0	100
5	96
10	95

was reacted at 0, 25, and 100 °C in 250 mL of deionized water at pH 6. The results are shown in Table I. The reaction at room temperature (25 °C) gave the best results for 2-acetylthiazolidine formation.

(2) *Molar Ratio of Starting Compounds.* This ratio was examined at 25 °C and pH 6. For molar ratios of cysteamine and methyl glyoxal (cysteamine/methyl glyoxal) in the range from 1 to 1000, the yield of 2-acetylthiazolidine was essentially constant at about 98%. On the basis of these two preliminary experiments, cysteamine was reacted with samples of interest at 25 °C and pH 6, and the amount of cysteamine used always exceeded 10 times the amount of methyl glyoxal estimated to be in the samples. Because some carbonyl compounds in the food samples were expected to compete with the methyl glyoxal for the available cysteamine, enough cysteamine was added to react with such carbonyl compounds as well as with all methyl glyoxal.

Preparation of Calibration Curves for Methyl Glyoxal Analysis. Cysteamine hydrochloride (0.75 g) was reacted with various amounts of methyl glyoxal (1–7.5 mg) in 250 mL of deionized water at 25 °C and pH 6. Each reaction mixture was extracted with 70 mL of dichloromethane by a liquid–liquid continuous extractor for 6 h. After removal of water from the extracts, 10 μL of *N*-methylacetamide standard solution was added to each extract prior to GC analysis. The GC peak area ratio of 2-acetylthiazolidine to the standard was plotted against quantity of methyl glyoxal in the original solution.

Recovery Efficiency of 2-Acetylthiazolidine in a D-Glucose Solution. Cysteamine hydrochloride (0.75 g) was reacted with 0.57 mg of methyl glyoxal in 250 mL of aqueous solutions containing 0, 5, and 10% of D-glucose at 25 °C and pH 6 to determine possible interference of D-glucose in 2-acetylthiazolidine formation. The results are shown in Table II.

Analysis of Methyl Glyoxal in Various Commercial Foods. *Soy Sauce.* Cysteamine hydrochloride (0.75 g) was added to 200 mL of soy sauce in a 500-mL Erlenmeyer flask, and the solution was immediately adjusted to pH 6 with 6 N NaOH. The solution was stirred for 30 min by a magnetic stirrer and extracted with 70 mL of dichloromethane for 6 h on a liquid-liquid continuous extractor.
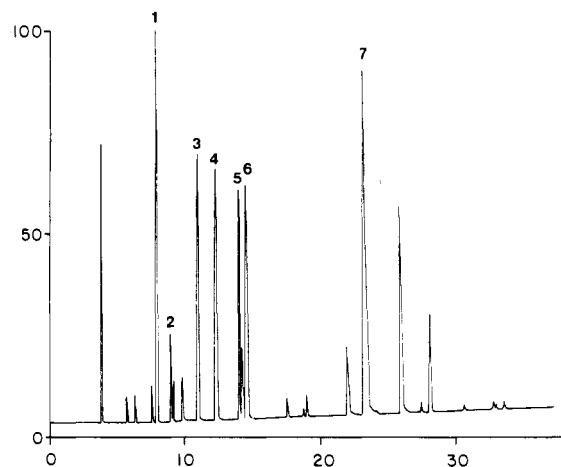

Figure 2. Gas chromatogram of the extract of cysteamine-treated soy sauce: peak 1, 2-methylthiazolidine; peak 2, thiazolidine; peak 3, 2-isopropylthiazolidine; peak 4, *N*-methylacetamide (internal standard); peak 5, isobutylthiazolidine; peak 6, unknown thiazolidine derivative; peak 7, 2-acetylthiazolidine.

The extract was dried over anhydrous sodium sulfate for 12 h. After removal of the sodium sulfate, 10 μL of *N*-methylacetamide standard solution was added to the solution as a GC internal standard. Finally, the extract was analyzed for 2-acetylthiazolidine by GC.

Soybean Paste. Soy bean paste (20 g) was dissolved in 200 mL of deionized water, and 0.75 g of cysteamine hydrochloride was added to this solution. The rest of the procedure was the same as that for the soy sauce.

Brewed Coffee. Regular or decaffeinated coffee (80 g) was added to 1 L of boiling water. After 10 min, each sample was filtered and 0.75 g of cysteamine hydrochloride was added to the filtrate. The rest of the procedure for each solution was the same that for the soy sauce.

Instant Coffee, Cocoa, Instant Tea, Maple Syrup, and Nonfat Dry Milk. Instant coffee (3 g), cocoa (5 g), instant tea (5 g), maple syrup (10 g), and nonfat dry milk (8 g) were each dissolved in separate 250-mL portions each of hot deionized water, and then 0.75 g of cysteamine hydrochloride was added to each solution. The rest of the procedure for each solution was the same as that for the soy sauce.

Cola, Wine, Beer, Orange Juice, Tomato Juice, Root Beer, and Apple Juice. Cysteamine hydrochloride (0.75 g) was added to 250 mL each of cola, wine, beer, orange juice, tomato juice, root beer, and apple juice. The rest of the procedure for each solution was the same as that for the soy sauce.

RESULTS AND DISCUSSION

When a food sample is treated with cysteamine, some food constituents such as carbohydrates may interfere with 2-acetylthiazolidine formation. D-Glucose was chosen to examine such possible interference by reduced sugars. Recovery of 2-acetylthiazolidine was lowered about 5% in the presence of 10% D-glucose. The quantity of D-glucose (139 mmol), however, was about 10000 times that of methyl glyoxal (0.0079 mmol) in the 10% D-glucose solution. Food constituents such as D-glucose apparently do not interfere significantly with 2-acetylthiazolidine formation. The average recovery of methyl glyoxal from either brewed coffee, instant tea, or cola was greater than 95% on the basis of three analyses for each food at the 1 ppm level.

The results of the methyl glyoxal analysis of foods are shown in Table III. Gas chromatograms of the extracts from cysteamine-treated soy sauce and from untreated soy

Table III. Results of Methyl Glyoxal Analysis in Foods and Calculated Amounts of Methyl Glyoxal Intake for Each Food When Consumed

food	amt methyl glyoxal, ppm	amt foods per serving	methyl glyoxal intake per serving, μg
		Group I	
brewed coffee	25	3/180 g/mL[a]	75.6
decaff brewed coffee	47	3/180	140.4
inst coffee	23	1/180	22.7
cocoa	1.2	4/180	4.9
inst tea	2.4	0.3/180	0.7
nonfat dry milk	1.4	22.7/240[b]	31.2
soy sauce A	7.6	c	c
soy sauce B	3.0	c	c
soy bean paste (Miso)	0.7		
		Group II	
cola A	0.23	354 mL/can	81.4
root beer A	0.76	354 mL/can	269.0
beer A	0.08	355 mL/can	29.7
wine (white)	0.11	100 mL/glass	11.0
apple juice	0.26	300 mL/glass	78.0
orange juice	0.04	354 mL/can	14.2
tomato juice	0.06	177 mL/can	11.3
maple syrup	2.5	c	c

[a] One cup.   [b] One glass.   [c] Not calculated.
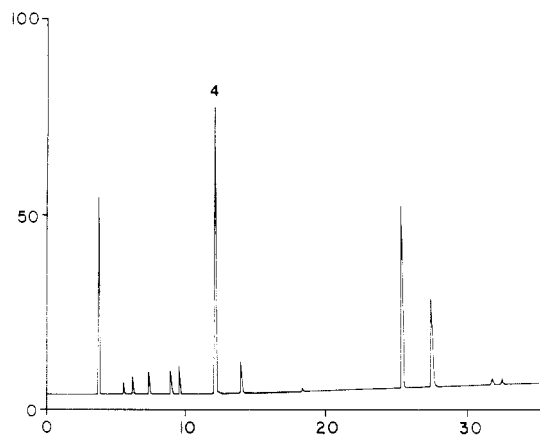
Figure 3. Gas chromatogram of the extract of untreated soy sauce: peak 4, N-methylacetamide (internal standard).

sauce are shown in Figures 2 and 3. The additional peaks in Figure 2 represent thiazolidines derived from aldehydes. Certain aldehydes (acetaldehyde, n-propanal, 2-methylpropanal, 3-methylbutanal) were found in soy sauce previously (Nunomura et al., 1976). Methyl glyoxal, however, has never before been reported in soy sauce or soybean paste. The content of methyl glyoxal in soybean paste was considerably lower than that of soy sauce even though they are prepared by similar procedures. This may be due to the difference of fermentation periods.

Coffee contained the largest quantity of methyl glyoxal among the food samples tested. Kasai et al. (1982), who analyzed methyl glyoxal as 2-methylquinoxaline using a GC equipped with a packed column, reported that the amounts of methyl glyoxal found in one cup of instant coffee and in one cup of brewed coffee were 100–150 and 470–730 μg, respectively. They estimated that one cup of coffee was 100 mL and required 1 g of instant coffee or 8 g of ground coffee beans to prepare. In the present study, one cup of instant coffee (1 g/100 mL) and one cup of brewed coffee (8 g/100 mL) contained 12.6 and 42–78 μg of methyl glyoxal, respectively. These values are 1 order of magnitude less than those just noted from Kasai et al. A difference in pH between the two experiments could

account for the 10-fold difference in recoveries. When we analyzed coffees treated with cysteamine at pH 8, for example, recoveries of methyl glyoxal from each cup of instant coffee and of brewed coffees were 430 and 540–620 ppm, respectively. Calculated values of methyl glyoxal in one cup of coffee under these conditions were 238–1030 μg. Kasai et al. did not indicate the pH or pH dependence of their methyl glyoxal recoveries, but if the pH was near 7, the results of our experiments are comparable.

As with coffee, more methyl glyoxal was recovered from soy sauce at pH 8 than at pH 6. The same trend, more or less, was observed in the other food samples. At a lower pH such as 6, methyl glyoxal may exist largely as a polymer or as a complex with an amino group of a large molecule such as a protein, but at a higher such as 8, some methyl glyoxal may be released from polymers or complexes. At pH 8, some methyl glyoxal may also be produced from sugars present in a food sample because sugar fragmentation occurs more readily under basic conditions. When the pHs of sample solutions were increased above 7, however, recovery of methyl glyoxal became inconsistent and was not reproducible. This may be due to inconsistent formation of methyl glyoxal from sugars or other unknown factors.

Foods containing a caramelized sugar such as cola and maple syrup were expected to have more methyl glyoxal than foods without, but at pH 6 the amount of methyl glyoxal detected in these foods with sugar was less than that of coffee or soy sauce. Further study is necessary to clarify the pH dependence of methyl glyoxal recovery from a food sample at pH higher than 7. When the pH of the sample solution was no greater than 6, on the other hand, the recovery of methyl glyoxal was consistent and reproducible. Moreover, in the range pH 4–6, the recovery efficiency of methyl glyoxal was highest (Figure 1). Therefore, the data from pH 6 are reported. Fortunately, most food items analyzed were already slightly acidic.

The samples examined in this study can be divided into two groups. One group is foods consumed without additional water (group I), and the other group is foods consumed with the addition of a certain amount of water (group II). Because methyl glyoxal is a proven mutagen, the amount of it consumed with various foods is important. Therefore, Table III reports the calculated intakes of methyl glyoxal in typical food portions in μg rather than in ppm for the food items tested. No intake of methyl glyoxal was calculated for soy sauce, however, because of wide variations in the amounts used by different individuals. The proposed method of using the thiazolidine derivative for methyl glyoxal determination proved successful. In two papers that reported the determination of methyl glyoxal using o-phenylenediamine (Moree-Testa and Saint-Jalm, 1981; Kasai et al., 1982), methyl glyoxal was reacted with o-phenylenediamine to give methylquinoxaline, which was then analyzed by GC. Comparing the present method to the o-phenylenediamine method is difficult because the pH dependence of quinoxaline formation was not reported in either of the two earlier papers. The thiazolidine method, however, can determine all carbonyl compounds simultaneously in contrast to the o-phenylenediamine method, which can determine only dicarbonyl compounds. In addition, the present study applied fused silica capillary GC, which gives higher resolution than the packed column GC used for the o-phenylenediamine method and consequently provides more precise quantitative data.

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Alterations of Soybean Lecithin during Curd Formation in Cheese Making

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The alterations of soybean lecithin during curd formation in cheese making have been studied with radioactive phosphatidylcholines and phosphatidylethanolamines. It was found that most of the added mixture of radioactive glycerophospholipids was not changed when processing the milk. Minor proportions of the labeled phospholipids were metabolized by starter and *Penicillium* cultures. Radioactive lipophilic hydrolysis products and radioactive hydrophilic metabolites of microbial origin both derived from labeled glycerophospholipids that had been added to cheese milk were concentrated predominantly in whey.

It has been reported that in making cheese the yield of curd can be increased by adding phosphatidylcholines (lecithins) to the milk (Bily, 1981). The characteristic feature of this process is that lecithin is added at a level of 0.001–0.15% to milk prior to the precipitation of the curd. The lecithin may be added to cheese milk at any period of time before the point of coagulation is reached.

We have recently described the distribution of added soybean phospholipids between curd and whey as well as the formation of casein containing lecithin liposomes that can be built into the rennet jelly when making cheese (Wiechen et al., 1985). In the present paper we report the turnover of phosphatidylcholines and phosphatidylethanolamines in curd and whey during the first steps of Camembert cheese making. The conversion of added soybean phospholipids is of interest, not only from a technological point of view but also with regard to food legislation.

EXPERIMENTAL SECTION

Chemicals. [1-^{14}C]Linoleic acid (specific activity 2.03 GBq/mmol) was purchased from Amersham Buchler (D-3300 Braunschweig, FRG). Commercial soybean lecithin, Metarin K, containing 20–23% of phosphatidylcholines and 21–24% of phosphatidylethanolamines was a product of Lucas Meyer GmbH, D-2000 Hamburg, FRG.

Determination of Radioactivity. Solutions were mixed with Aquasol-2 (NEN-Chemicals, D-6072-Dreieich, FRG), and radio activity was determined by liquid scintillation counting in a Tri-Carb C 2425 instrument (Packard Instruments Co., Downers Grove, IL 60515).

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The distribution of radioactive fractions of thin-layer chromatograms was determined with a Berthold TLC scanner, Model LB 2760, or with a Berthold Automatic TLC linear analyzer, Model LB 2832, in combination with a data acquisition system LB 500 (BF-Vertriebsgesellschaft, D-7547 Wildbad, FRG). After two-dimensional TLC, labeled fractions were detected with the automatic TLC linear analyzer system and evaluated by using a perspective TLC program. Radio gas chromatography was carried out in a Perkin-Elmer F 22 instrument (Perkin-Elmer Bodenseewerk, D-7770 Überlingen, FRG) equipped with thermal conductivity detectors. Methyl esters of fatty acids were analyzed on a glass column, 1.8 m × 4 mm, packed with 10% Silar 5 CP on Gas Chrom Q, 100–120 mesh (Applied Science Laboratories, State College, PA 16801) at 180 °C with helium as the carrier gas at a flow rate of 40 mL/min. Radioactivity in the carrier gas effluent was monitored in a Packard gas proportional counter, Model 894 (Packard Instruments Co.), combined with a Spectra Physics SP 4270 integrator (Spectra-Physics, D-6100 Darmstadt, FRG).

Cell Suspension Cultures of Soya. Heterotrophic cell suspension cultures of soya were propagated in B$_5$ medium (Gamborg et al., 1968) containing 2 × 10^{-6} M (2,4-dichlorophenoxy)acetic acid. The soya cells were shaken in the dark at 25 °C and subcultured every 10–14 days.

Preparation of Radioactively Labeled Phosphatidylcholines and Phosphatidylethanolamines. Heterotrophic soya cells, 10 g of cells/30 mL of preconditioned medium, were preincubated for 1 h under anaerobic conditions (argon atmosphere) and then added to 370 kBq (1.0 μmol) of [1-^{14}C]linoleic acid in 0.02 mL of 80% aqueous ethanol–diethyl ether (1:2, v/v). The cells were incubated at 25 °C for 0.5 h under anaerobic conditions. The cells were collected by repeated centrifugation and washing with 0.1 M sodium phosphate–potassium phosphate buffer of pH 6.0. The pellets were suspended in 2 mL of 2-propanol and heated in closed tubes at 100 °C for 10 min. The cells were homogenized, and the lipids were extracted according