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Formation of 4(5)-Methylimidazole and Its Precursors, α-Dicarbonyl Compounds, in Maillard Model Systems

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ABSTRACT: Glyoxal, methylglyoxal, and diacetyl formed from sucrose alone and from a D-glucose/ammonia Maillard model system were analyzed by gas chromatography. They are known as precursors of 4(5)-methylimidazole (MI). Glyoxal and methylglyoxal formed more in acidic conditions than in basic conditions, whereas diacetyl formed the most at the highest basic condition of pH 12. Glyoxal formation from sucrose ranged from 0.33 to 32.90 μg/g under four different time and temperature conditions. Amounts of glyoxal, methylglyoxal, and diacetyl formed in Maillard model systems ranged from 2.98 to 46.12 μg/mL, from 8.27 to 156.61 μg/mL, and from 14.94 to 1588.45 μg/mL, respectively. 4(5)-MI formation in the same model systems ranged from 28.56 to 1269.71 μg/mL. Addition of sodium sulfite reduced formation of these chemicals significantly. Total α-dicarbonyl compounds in 12 commercial soft drinks ranged from 5.75 to 50.72 μg/mL. 4(5)-MI was found in levels ranging from 1.76 to 28.11 ng/mL in 10 commercial soft drinks.

KEYWORDS: *diacetyl, glyoxal, Maillard reaction, methylglyoxal, 4(5)-methylimidazole, soft drinks*

INTRODUCTION

4(5)-Methylimidazole [4(5)-MI] was determined to be a cancer-causing chemical by the National Toxicology Program in 2007.[1] However, some toxic chemicals, including 4(5)-MI, aromatic amines, polycyclic aromatic hydrocarbons (PAHs), and acrylamide, unavoidably form in foods under heat treatment.[2] Therefore, it is desirable to find a way to mitigate the formation of carcinogenic 4(5)-MI in foods and beverages during heat treatment. To investigate potential mitigation strategies, it is extremely important to know the specifics of 4(5)-MI formation mechanisms in foods and beverages.

As mentioned above, it is apparent that 4(5)-MI forms via the Maillard reaction. Numerous heterocyclic compounds including imidazoles, which are known to enhance cooked flavors, have been reported in Maillard reaction products.[3] However, the presence of 4(5)-MI in Maillard reaction systems has not received much attention from flavor chemists due to its noncharacteristic flavor.[4]

A recent review describes the biological and chemical properties of 4(5)-MI.[4] It reports the presence of 4(5)-MI in various foods and beverages, particularly in ones with caramel colors. However, details of its formation mechanisms have not been well established yet. Some mechanisms based on the results of experimental synthesis of 4(5)-MI were proposed. For example, 4(5)-MI was synthesized from a reaction of formaldehyde and glyoxal with ammonia.[5] Therefore, it was proposed that 4(5)-MI is formed from α-dicarbonyl compounds with ammonia. In fact, when methylglyoxal and formaldehyde were heated in an aqueous solution with ammonium hydroxide, 5.45 mg/mL of 4(5)-MI was formed.[6] This study also demonstrated that 4(5)-MI was formed in Maillard reaction systems consisting of D-glucose and ammonia (NH_4OH). The so-called caramel color III is prepared from a sugar and ammonia and is used in alcoholic beverages. Caramel color IV is manufactured from a sugar and ammonia with a sulfite-containing chemical and is used heavily in soft drinks.[7] These reports suggest that 4(5)-MI is formed by the Maillard reaction and also that it formed from the reaction of α-dicarbonyl compounds and ammonia.

The Maillard reaction occurs between carbonyl compounds and nitrogen-containing compounds such as amino acids. It is also called the nonenzymatic amino−carbonyl browning reaction. The question is exactly where these carbonyl compounds and nitrogen-containing compounds come from in foods. It is generally recognized that nitrogen comes from an amino acid via Strecker degradation.[8] The sources of carbonyl compounds are relatively complicated. One of the sources of carbonyl compounds is lipids, which produce many low molecular weight carbonyl compounds, including α-dicarbonyl compounds, upon oxidative degradation.[9] One recent study demonstrated that glyoxal, methylglyoxal, and diacetyl formed from heated lipids (butter, margarine, and safflower oil) upon heat treatment.[10] However, 4(5)-MI may mainly be produced by reaction between carbonyl compounds from a sugar and ammonia from an amino acid because there have been many reports on the formation of 4(5)-MI in various sugar/amino acid Maillard model systems.[4]

In the present study, formation of α-dicarbonyl compounds from sugars and 4(5)-MI from D-glucose and ammonia model systems was investigated to elucidate the formation mechanisms of 4(5)-MI in foods and beverages.

MATERIALS AND METHODS

Chemicals and Reagents. D-Glucose, ammonium hydroxide solution (29%), sodium sulfite, glyoxal (ethanedial), 2-methylglyoxal (2-oxopropanal), diacetyl (2,3-butanedione), *o*-phenylenediamine dihydrochloride, quinoxaline, 2-methylquinoxaline, and 2,3-dimethylquinoxaline were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

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Authentic 4(5)-MI was bought from Aldrich Chemical Co. (Milwaukee, WI, USA), and its standard stock solution was prepared in HPLC grade methanol at a concentration of 100 mg/L. The solution was stored in the dark at 4 °C until used. Organic solvents were from Fisher Scientific (Fair Lawn, NJ, USA). Commercial soft drinks and sucrose (cane sugar) were purchased from a local market.

Sample Preparations for Analysis of α-Dicarbonyl Compounds in Heated Sugars. Cane sugar (35 g) was dissolved in 50 mL of deionized water in a 250 mL swing-top bottle, and the pH was adjusted to 2, 6, 8, or 12. Each aqueous sample was heated at 120 or 160 °C for 2 or 4 h in an oven. After the sample had cooled to room temperature, derivatizing reagent *o*-phenylenediamine dihydrochloride (1.5 g) was added, and the pH was adjusted to 12; then the sample solution was stirred for 2 h. The reaction mixture (5 mL) was passed through a C_{18} SPE cartridge (1000 mg × 6 mL, Agilent, Lake Forest, CA, USA). A cartridge was preconditioned twice with 5 mL of ethyl acetate. The remaining materials in the cartridge were eluted with a 5 mL portion of ethyl acetate twice under reduced pressure. The elutate was condensed with a purified nitrogen stream, and then 20 μL of a 2-methylpyrazine (500 mg/L)−ethyl acetate solution was added as a GC internal standard. The sample solution was adjusted to exactly 5.0 mL in volume with ethyl acetate

An Agilent model HP 6890 series GC equipped with a 30 m × 0.25 mm i.d. DB-WAX fused silica capillary column and an nitrogen−phosphorus detector (NPD) was used for the quantitative analysis of α-dicarbonyl compounds (glyoxal, methylglyoxal, and diacetyl) as quinoxalines. The oven temperature was held at 40 °C for 2 min and then programmed to rise to 170 °C at 4 °C/min and held for 15 min. Helium carrier gas rate was 1.5 mL/min. The injector was operated at 260 °C with splitless mode. The detector temperature was 300 °C.

Sample Preparations for Analysis of α-Dicarbonyl Compounds and 4(5)-MI in Maillard Reaction Systems. Aqueous solutions (30 mL) containing three levels of D-glucose and ammonium hydroxide, 1.0 M each (group I), 0.5 M each (group II), and 0.1 M each (group III), and sodium sulfite (0, 0.1, 0.2, 0.5, 0.7, or 1.0 M) were heated in a swing-top bottle at 150 °C for 2 h in an oven. The reaction samples were cooled to room temperature and then stored at 4 °C until used.

For α-dicarbonyl analysis, a reaction sample was diluted 2−25-fold with deionized water for solid-phase extractions (SPE). Diluted samples (3 mL) were mixed with 2 mL of *o*-phenylenediamine dihydrochloride solution (100 mg/10 mL), and the pH was adjusted to 12. After the solution had been stirred for 2 h at room temperature, the reaction solution was passed through a C_{18} SPE cartridge (1 g × 6 mL, Agilent), and the aqueous eluate was passed through a second cartridge connected in series. Retained materials in the two cartridges were eluted with ethyl acetate (5 mL each). The ethyl acetate eluates from the two cartridges were combined and dried over anhydrous sodium sulfate. After the eluate sample had been adjusted to exactly 10 mL in volume with ethyl acetate, 20 μL of 1-methylpyrazole solution (10 mg/mL) was added as a GC internal standard. The sample solution was analyzed for glyoxal, methylglyoxal, and diacetyl as a corresponding quinoxaline derivative by GC-NPD. The experiments were repeated three times.

For 4(5)-MI analysis, reaction samples were diluted 5−50-fold with deionized water for SPE. After the pH of a diluted sample (5 mL) had been adjusted to 12, it was passed through a C_{18} SPE cartridge, and the aqueous eluate was passed through a second cartridge connected in series. Retained materials in the two cartridges were eluted with methanol (10 mL each). The methanol eluates from the two cartridges were combined and then dried over anhydrous sodium sulfate. After the eluate sample had been condensed to approximately 2.7 mL in volume with a purified nitrogen stream, the volume was adjusted to exactly 3 mL with methanol. An internal standard, 60 μL of 1-benzylimidazole solution (0.5 mg/mL), was added for analysis of 4(5)-MI by GC-NPD. The experiments were repeated three times.

Sample Preparations for α-Dicarbonyl Compounds and 4(5)-MI Analysis in Commercial Soft Drinks. Commercial soft drink samples (3 mL each) were prepared using the same procedures as the samples from the Maillard reaction systems without dilution. The experiments were repeated three times.

Analysis of α-Dicarbonyl Compounds (Glyoxal, Methylglyoxal, and Diacetyl) and 4(5)-MI in the Samples. Identification of quinoxaline (glyoxal), methylquinoxaline (methylglyoxal), 2,3-dimethylquinoxaline (diacetyl), and 4(5)-MI was performed by comparison with the Kovats gas chromatographic retention index *I*[11] and by the MS fragmentation pattern of each component compared with those of authentic chemicals. Quantitative analysis was conducted by the GC internal standard method.[12]

For analysis of α-dicarbonyl compounds, an Agilent model 6890 GC equipped with a 30 m × 0.25 mm i.d. (*df* = 0.25 μm) DB-WAX bonded-phase fused-silica capillary column (Agilent, Folsom, CA, USA) and an NPD was used. The helium carrier gas flow rate was 1.5 mL/min at splitless mode. The injector and detector temperatures were 260 and 300 °C, respectively. The oven temperature was held at 40 °C for 2 min and then programmed to 170 °C at 4 °C/min and held for 15 min.

For analysis of 4(5)-MI, an Agilent model 6890 GC equipped with a 60 m × 0.25 mm i.d. (*df* = 0.25 μm) DB-WAX bonded-phase fused-silica capillary column (Agilent) and an NPD was used. The helium carrier gas flow rate was 1.5 mL/min at splitless mode. The injector and detector temperatures were 260 and 300 °C, respectively. The oven temperature was programmed from 80 to 170 °C at 10 °C/min and held for 10 min.

Recovery Efficiency Test for α-Dicarbonyl Compounds. A standard calibration curve for the three quinoxaline derivatives was prepared using various concentrations of ethyl acetate solutions (0.5, 1, 5, 10, 50, 100, 500, and 1000 μg/mL). Each curve was prepared according to the internal standard method with 2-methylpyrazine (500 μg/mL) as an GC internal standard.

An aqueous solution (5 mL) containing 2 mL of derivatizing reagent, *o*-phenylenediamine hydrochloride (10 mg/mL), was spiked with glyoxal, 2-methylglyoxal, and diacetyl (1, 10, 100, or 500 μg/L), and the pH of the solution was adjusted to 12. The solution was stirred for 2 h at room temperature. The reaction sample was prepared for analysis of the three derivatives (quinoxaline, methylquinoxaline, and 2,3-dimethylquinoxaline) with SPE and analyzed by using GC-NPD according to the method described above.

Recovery Efficiency Test for 4(5)-MI. A standard calibration curve for 4(5)-MI was prepared using different concentrations of standard 4(5)-MI in methanol (0.25, 0.5, 1, 2.5, 5, 10, 25, and 50 μg/mL). The recovery efficiency of 4-MI was examined using 1 and 10 μg/mL aqueous solutions (5 mL). The testing samples were prepared with SPE and analyzed for 4(5)-MI with GC-NPD according to the method described above.

Analysis of 4(5)-Methylimidazole in Commercial Soft Drinks by LC-MS. 4(5)-MI in one commercial soft drink was analyzed by LC-MS to confirm the gas chromatographic method to be used in the present study, which has been reported previously.[6] A Hewlett-Packard 1100 liquid chromatograph interfaced to an Applied Biosystems API 2000 MS/MS via an atmospheric pressure chemical ionization (APCI) source operating in the positive ion mode at 400 °C with nitrogen gas was used. MS was equipped with a 100 × 4.6 mm (3.0 μm i.d.) Varian Polaris RP column (5 μm particle size) (Varian, Walnut Creek, CA, USA). The injection volume of each sample was 5 μL. The mobile phase was water (15 mmol ammonium hydroxide, solvent A) and acetonitrile (15 mmol ammonia, solvent B). A linear gradient from A/B = 98/2 at 0−3 min to 60/40 at 10−13 min and to 98/2 at 15−25 min was used with a flow rate of 0.5 mL/min. Under these conditions, 4(5)-methylimidazole eluted at 6.8 min. The mass spectrometer was operated in selective ion monitoring mode (SIM) to observe the transition of *m/z* 83 to *m/z* 56 for 4(5)-MI.

Statistical Processing. The results of the present study were averaged, and the comparison between experimental groups was drawn through an ANOVA analysis based on the SAS system. After the ANOVA analysis, the level of significance was computed using Duncan's multiple-range test at $\alpha = 0.05$.

Table 1. Recovery Efficiencies of α-Dicarbonyl Compounds from Aqueous Solutions as a Corresponding Quinoxaline Derivative

	amount spiked			
	1 μg/mL	10 μg/mL	100 μg/mL	500 μg/mL
glyoxal	63.8 ± 4.8	106.7 ± 1.0	70.9 ± 9.7	70.4 ± 6.7
methylglyoxal	90.3 ± 3.3	87.9 ± 2.4	93.8 ± 2.6	90.8 ± 2.4
diacetyl	106.3 ± 8.7	75.6 ± 12.7	90.8 ± 1.0	82.9 ± 6.1

RESULTS AND DISCUSSION

Recovery efficiency of α-dicarbonyl compounds is shown in Table 1. Recovery efficiencies of α-dicarbonyl compounds ranged from 63.8 to 106.7% for glyoxal, from 87.9 to 93.8% for methylglyoxal, and from 75.6 to 106.3% for diacetyl. The linearity value (R^2) of a standard curve for quantitative analysis was 0.9985−0.9999 for quinoxaline, 0.9992−0.9999 for 2-methylquinoxaline, and 0.9977−0.9999 for 2,3-dimethylquinoxaline. The limit of detection (LOD) was 0.5 ng for glyoxal as quinoxaline, 1.0 ng for methylglyoxal as 2-methylquinoxaline, and 0.3 ng for diacetyl as 2,3-dimethylquinoxaline. The limit of quantitation (LOQ) was 1.32 ng for glyoxal as quinoxalines, 1.04 ng for methylglyoxal as methylquinoxaline, and 1.54 ng for diacetyl as 2,3-dimethylquinoxaline.

Recovery efficiency of 4(5)-MI was 100.33 ± 2.92% at the level of 1 μg/mL and 90.46 ± 4.40% at the level of 10 μg/mL from the aqueous solutions. The linearity value of a standard curve for quantitative analysis was 0.9997. The LOD was 0.2 ng and the LOQ was 0.7 ng for 4-methylimidazole. A C_{18} silicic acid SPE, which is slightly acidic, trapped both 4(5)-MI and quinoxalines, which are slightly basic, efficiently, and recovery efficiencies of these chemicals were satisfactory in the present study.

α-Dicarbonyl Compounds Formed in Heated Sucrose. Figure 1 shows the amounts of three α-dicarbonyl compounds found in sucrose heated at 120 °C in an aqueous solution under four different pH values. Generally, amounts of α-dicarbonyl compounds found varied over the pH values. Glyoxal and methylglyoxal formed more in acidic conditions than in basic conditions, whereas diacetyl formed the most at the highest basic condition of pH 12. Glyoxal formation ranged from 0.33 ± 0.03 μg/g (2 h, pH 12) to 32.90 μg ± 5.31/g (2 h, pH 2) under the four different conditions. The results indicated that more glyoxal formed at the lower pH. Methylglyoxal formed in the greatest amount of the three α-dicarbonyl compounds, ranging from 9.85 ± 1.17 μg/g (2 h, pH 8) to 245 ± 41.60 μg/g (2 h, pH 2). Methylglyoxal reduced significantly after 4 h of heating, however, suggesting that it degrades during prolonged exposure to heat. On the other hand, glyoxal increased slightly after 4 h of heating. In the case of diacetyl, amounts formed ranged from 0.53 ± 0.04 μg/g (2 h, pH 8) to 14.83 ± 1.82 μg/g (4 h, pH 12).

Figure 2 shows the amounts of α-dicarbonyl compounds found in aqueous sucrose solution heated at 160 °C for 2 and 4 h under different pH values. The range of formation levels obtained from three α-dicarbonyl compounds was from 12.47 ± 0.54 μg/g (4 h, pH 12) to 26.14 0.96 μg/g (4h, pH 6) from glyoxal, from 39.18 ± 7.11 μg/g (4 h, pH 2) to 321.87 ± 56.72 μg/g (2 h, 12 pH) from methylglyoxal, and from 27.96 ± 3.84 μg/g (2 h, pH 8) to 92.45 ± 12.26 μg/g (4 h, pH 8) from diacetyl. The conditions that gave the highest level of total α-dicarbonyl compounds were pH 2, 120 °C, and 2 h (282.36 μg/g). These results indicated that there is no clear rule for sugar degradation under these conditions but, generally, that some

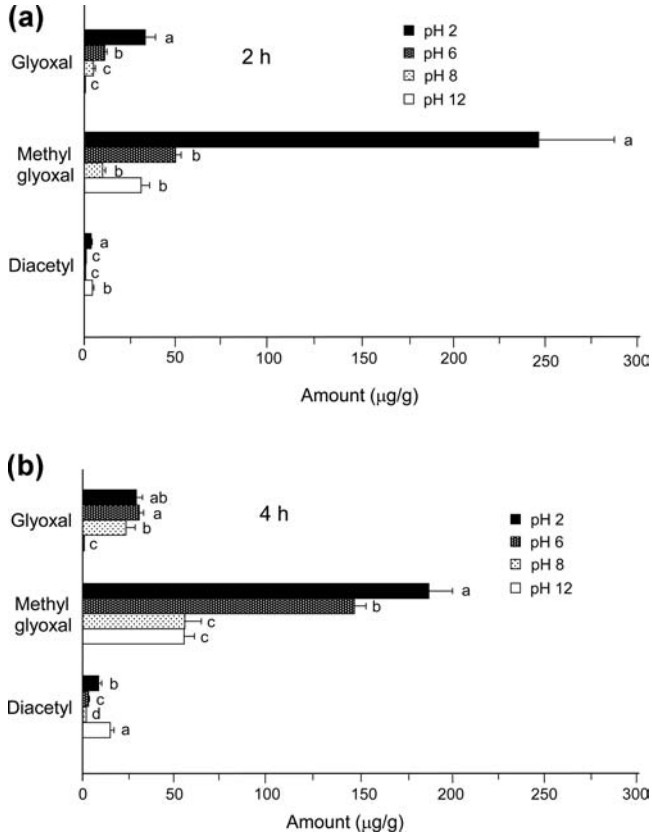

Figure 1. Amounts of α-dicarbonyl compounds found in sucrose heated at 120 °C for 2 and 4 h in an aqueous solution under four different pH values. Values are the mean ± SD (n = 3).

α-dicarbonyl compounds formed may degrade during prolonged heat treatment.

It has been known since the 1960s that sugars degrade into many low molecular weight flavor compounds including glyoxal, methylglyoxal, and diacetyl.[13] Figure 3 shows the proposed formation pathways of glyoxal, methylglyoxal, and diacetyl from sucrose. In this pathway, the first step is tautomerization of a free sugar group, followed by cleavage at keto−enol bonding in acidic or alkaline solution.[14] This may explain the higher levels of α-dicarbonyl compound formation in acidic or basic pH than in neutral pH found in the present study. In addition to the formation pathways of α-dicarbonyl compounds shown in Figure 3, there is a pathway in which a sugar produces various carbonyl radicals [$^{\bullet}C{=}O$, $^{\bullet}CHO$, $^{\bullet}C{=}O(CH_3)$], and these radicals subsequently combine to yield α-dicarbonyl compounds. This pathway could be proposed when the reaction systems absorb high levels of energy from high temperature or photoirradiation.[15] In fact, when acetaldehyde, acrolein, propanal, and acetone were irradiated by UV, 2.43−8.23 nmol/mg glyoxal and 2.17−9.20 nmol/mg methylglyoxal were formed.[16] Moreover, this

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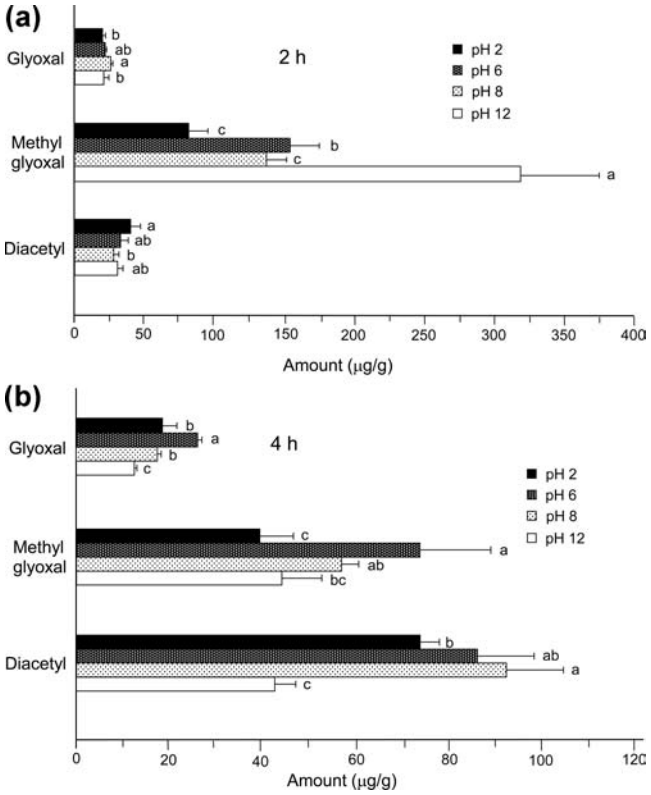

Figure 2. Amounts of α-dicarbonyl compounds found in sucrose heated at 160 °C for 2 and 4 h in an aqueous solution under four different pH values. Values are the mean ± SD (n = 3).

pathway would explain the inability to ascertain an appropriate formation rule under the heating conditions used in the present study.

α-Dicarbonyl Compounds Formed in Maillard Model Systems. Figure 4 shows the amounts of α-dicarbonyl compounds found in Maillard model systems consisting of D-glucose, ammonia, and sulfite. Generally, all three α-dicarbonyl compounds reduced with the addition of sulfite. Formation levels of glyoxal ranged from 6.99 ± 0.82 μg/mL (0 sulfite) to 2.98 ± 0.37 μg/mL (1.0 M sulfite) in group I (0.1 M D-glucose, 0.1 M ammonia), from 28.32 ± 0.89 μg/mL (0 sulfite) to 13.98 ± 0.77 μg/mL (0.7 M sulfite) in group II (0.5 M D-glucose, 0.5 M ammonia), and from 46.12 ± 3.335 μg/mL (0 sulfite) to 30.94 ± 2.37 μg/mL (1.0 M sulfite) in group III (1.0 M D-glucose, 1.0 M ammonia). Formation levels of methylglyoxal ranged from 128.91 ± 27.82 μg/mL (0.1 M sulfite) to 8.27 ± 0.31 μg/mL (1.0 M sulfite) in group I (0.1 M D-glucose, 0.1 M ammonia), from 160.05 ± 17.12 μg/mL (0 sulfite) to 15.98 ± 1.43 μg/mL (1.0 M sulfite) in group II (0.5 M D-glucose, 0.5 M ammonia), and from 156.61 ± 13.13 μg/mL (0 sulfite) to 51.28 ± 3,27 μg/mL (1.0 M sulfite) in group III (1.0 M D-glucose, 1.0 M ammonia). Formation levels of diacetyl ranged from 67.28 ± 3.99 μg/mL (0 sulfite) to 14.96 ± 1.81 μg/mL (1.0 M sulfite) in group I (0.1 M D-glucose, 0.1 M ammonia), from 602.63 ± 60.36 μg/mL (0 sulfite) to 79.76 ± 7.96 μg/mL (1.0 M sulfite) in group II (0.5 M D-glucose, 0.5 M ammonia), and from 1.588.45 ± 204.29 μg/mL (0 sulfite) to 314.39 ± 8.54 μg/mL (1.0 M sulfite) in group III (1.0 M D-glucose, 1.0 M ammonia). The addition of sulfite reduced the formation of glyoxal by 57%, that of methylglyoxal by 93%, and that of diacetyl by 78% in group I (0.1 M); that of glyoxal by 50%, that of methylglyoxal by 90%, and that of diacetyl by 87% in group II (05 M); and that of glyoxal by 33%, that of methylglyoxal by 67%, and that of diacetyl by 80% in group III (1.0 M). The results clearly indicate that the addition of sulfite reduces the formation of α-dicarbonyl compounds.

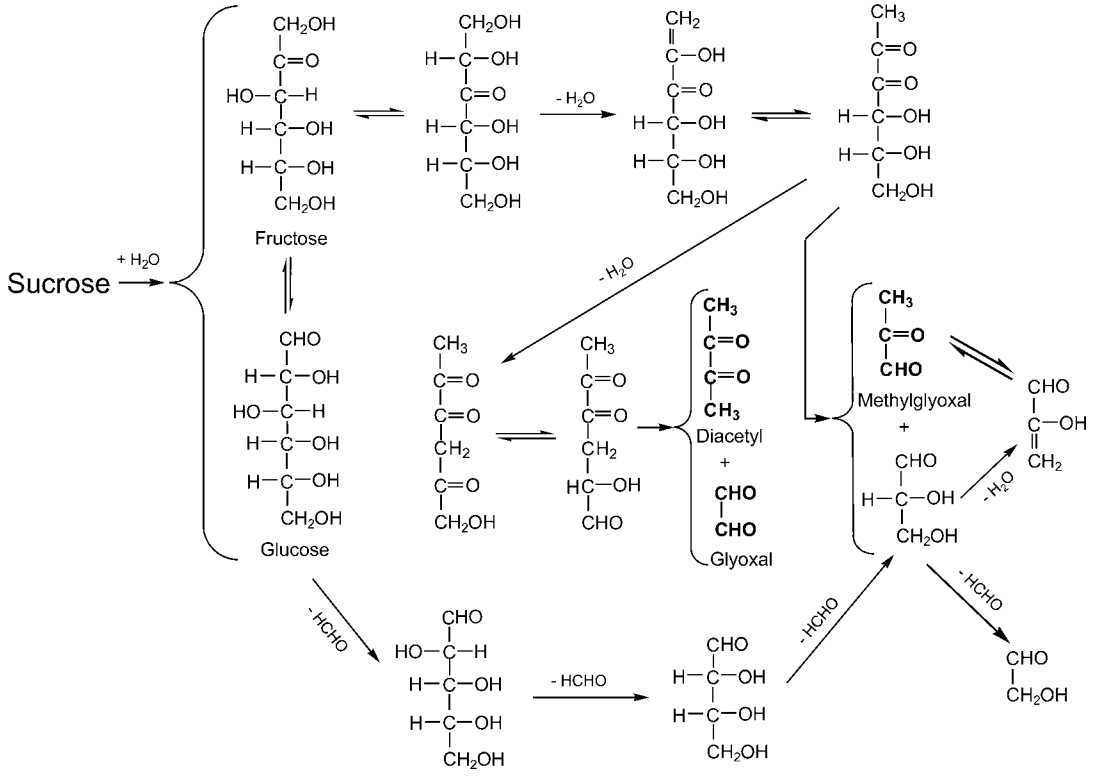

Figure 3. Proposed formation pathways of glyoxal, methylglyoxal, and diacetyl from sucrose.

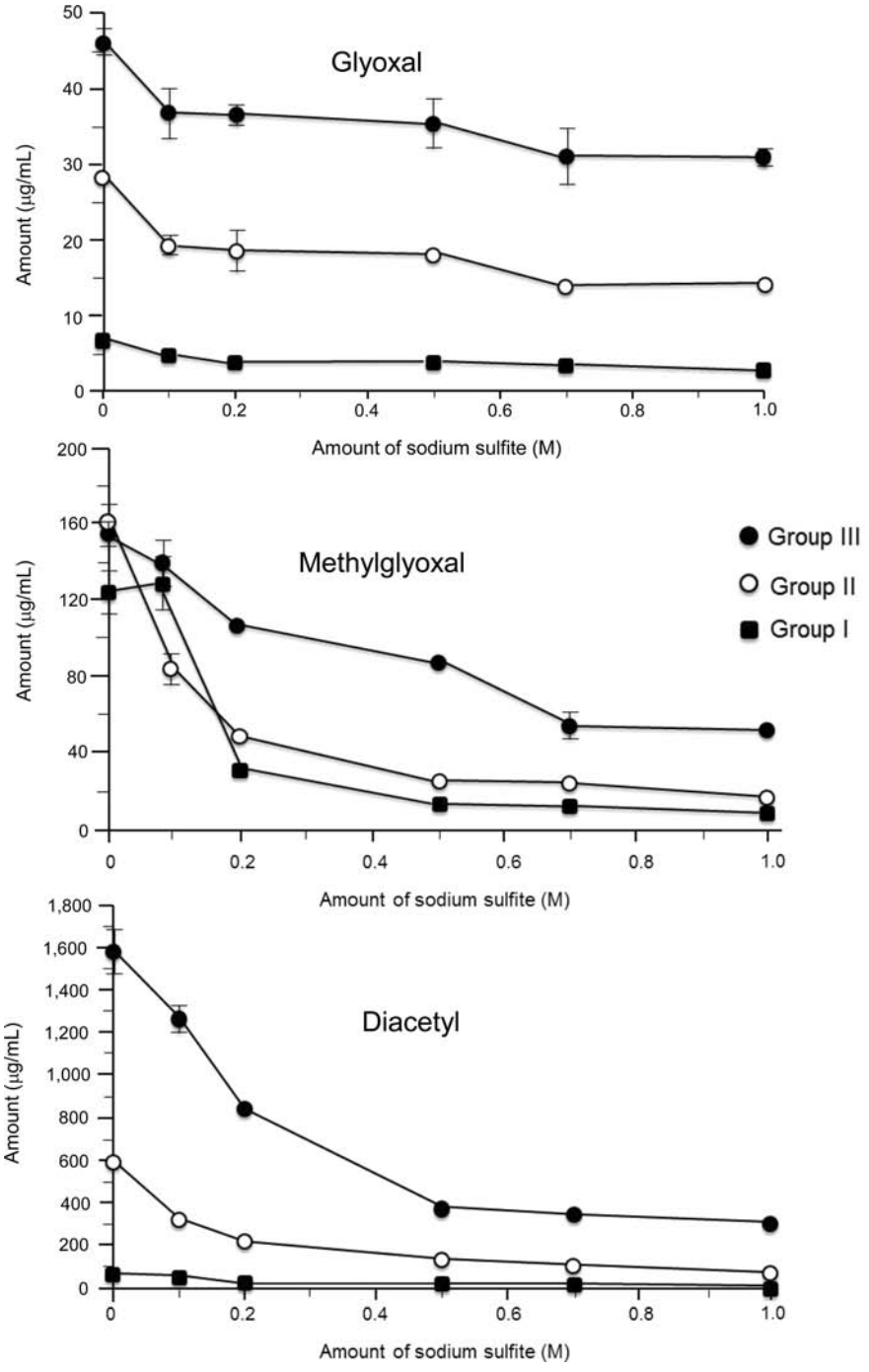

Figure 4. Amounts of α-dicarbonyl compounds found in Maillard model systems consisting of D-glucose, ammonia, and sulfite. Values are the mean ± SD (n = 3). SD values for group I (<1.00), group II (<5.00), and group III (<60.00) do not appear in the figure.

4(5)-MI Formed in Maillard Model Systems. There have been a several studies on the degradation of sugars to form α-dicarbonyl compounds in Maillard reaction systems in which an amino compound plays an important role in sugar degradation. An amino group has been proposed as a catalyst through Schiff base formation in sugar degradations.[17,18] For example, 11 α-dicarbonyl compounds were identified in a buffer solution of glucose/lysine heated at 50 °C for 7 days.[19] Also, catalytic activity of amino acids in glyoxal and methylglyoxal formation from different carbohydrates, including D-glucose, upon caramelization and Maillard reaction, have been observed.[20]

Figure 5 shows the amounts of 4(5)-MI found in Maillard model systems consisting of D-glucose, ammonia, and sulfite. As in the case of α-dicarbonyl compound formation, 4(5)-MI formation was reduced by the addition of sulfite. Formation levels of 4(5)-MI ranged from 52.78 ± 0.94 μg/mL (0 sulfite) to 28.56 ± 0.65 μg/mL (1.0 M sulfite) in group I (0.1 M D-glucose, 0.1 M ammonia), from 666.69 ± 39.32 μg/mL (0 sulfite) to 241.02 ± 19.65 μg/mL (1.0 M sulfite) in group II (0.5 M D-glucose, 0.5 M ammonia), and from 1269.71 ± 214.7 μg/mL (0 sulfite) to 739.53 69.39 μg/mL (1.0 M sulfite) in group III (1.0 M D-glucose, 1.0 M ammonia). The addition of sulfite reduced 4(5)-MI formation by 46% in group I, by

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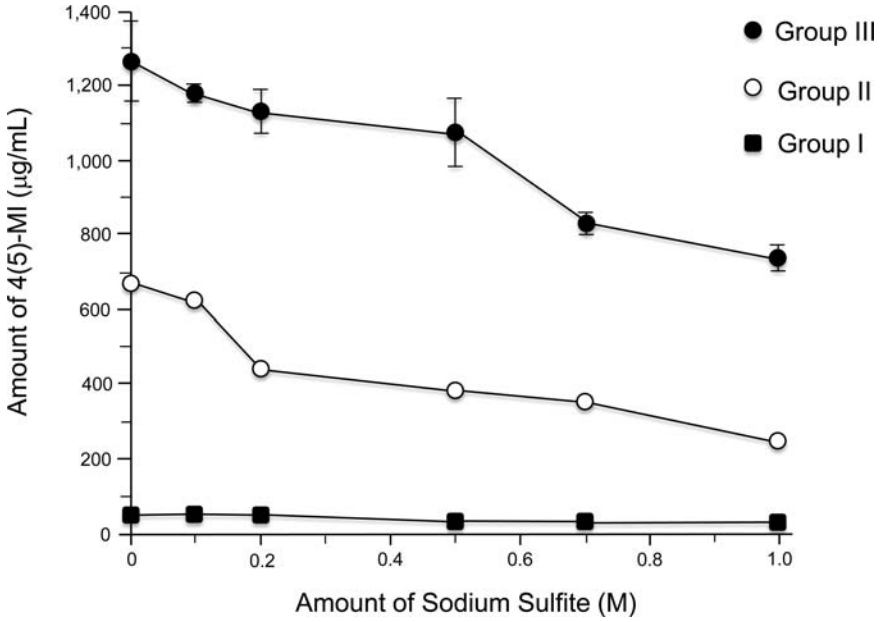

Figure 5. Amounts of 4(5)-MI found in Maillard model systems consisting of D-glucose, ammonia, and sulfite. Values are the mean ± SD (n = 3). SD values for group I (0.65−6.17) and group II (10.47−39.32) do not appear in the figure.

64% in group II, and by 42% in group III. The results also indicate that addition of sulfite reduces 4(5)-MI formation. This role of sulfite in 4(5)-MI formation in the same Maillard reaction systems was also previously reported.[7] Sulfite has been already used to manufacture caramel, in particular, the so-called caramel color IV, which is used widely in soft drinks such as colas.[21] Therefore, sulfite may be useful in suppressing the formation of 4(5)-MI as well as in obtaining preferable colors.

Figure 6 shows a typical proposed formation pathway of 4(5)-MI from α-dicarbonyl compounds prepared on the basis

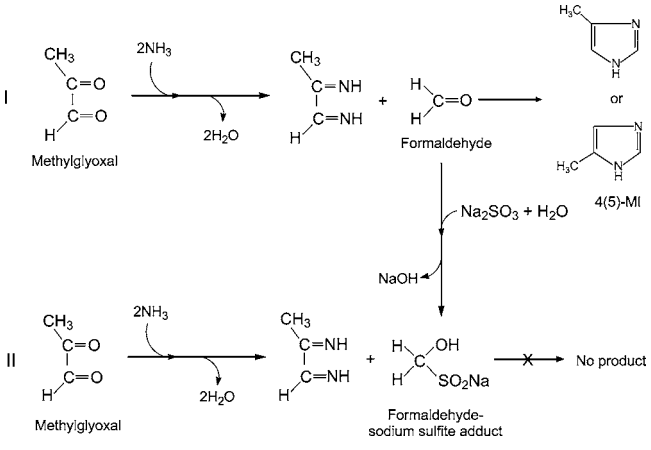

Figure 6. Proposed formation pathway of 4(5)-MI from α-dicarbonyl compounds prepared on the basis of the Debus−Radziszewski imidazole synthesis.

of the Debus−Radziszewski imidazole synthesis.[4] Pathway I shows the formation of 4- or 5-MI from methylglyoxal and formaldehyde, which forms from D-glucose according to Figure 3. The formaldehyde must be one of the reactants to form an imidazole ring, in which a C-2 atom is free from a substituent. It is known that sodium sulfite forms adduct with an aldehyde; this reaction was used conventionally for the determination of aldehydes.[22] Therefore, if formaldehyde was trapped with sodium sulfite as shown in pathway II, the formation of 4(5)-MI might be reduced. This phenomenon may explain the reduction of 4(5)-MI formation in the Maillard reaction systems reported in the previous study[21] and the present study.

α-Dicarbonyl Compounds and 4(5)-MI Found in Commercial Soft Drinks. Table 2 shows the amounts of glyoxal, methylglyoxal, diacetyl, and 4(5)-MI found in commercial soft drinks. Values are the mean ± SD (n = 3). In the case of soft drinks colored with caramel, levels of α-dicarbonyl compounds ranged from 0.57 ± 0.05 μg/mL (zero, brand II) to 16.04 ± 5.13 μg/mL (regular, brand I) for glyoxal, from 0.77 ± 0.01 μg/mL (diet, brand II) to 26.97 ± 10.08 μg/mL (regular, brand I) for methylglyoxal, and from 5.94 ± 1.05 μg/mL (regular, brand II) to 9.11 ± 1.43 μg/mL (zero, brand II) for diacetyl. It is obvious that regular drinks, which contain sugar, contained much higher levels of glyoxal and methylglyoxal than ones without sugar, suggesting that they were formed from sugar degradations as shown in Figure 3. On the other hand, differences in levels of diacetyl among three kinds of products (regular, diet, zero) were not significant, suggesting that diacetyl formation depends mainly on radical reactions.

In the case of colorless soft drinks, levels of α-dicarbonyl compounds ranged from 0.00 (zero, brand IV) to 13.57 ± 2.03 μg/mL (regular, brand III) for glyoxal, from 0.24 ± 0.07 μg/mL (diet, brand IV) to 20.45 ± 4.40 μg/mL (regular, brand IV) for methylglyoxal, and from 3.48 ± 0.70 μg/mL (regular, brand IV) to 11.00 ± 2.49 μg/mL (diet, brand III) for diacetyl. As in the case of colored soft drinks, regular drinks contained much higher levels of glyoxal and methylglyoxal than sugar-free drinks, suggesting that these α-dicarbonyl compounds are formed from sugars via non-Maillard reactions. A similar trend in levels of diacetyl as in the case of colored soft drinks was observed. A diet version of brand III exhibited the highest level of diacetyl followed by a regular version of brand III, suggesting that this drink contained some chemicals which produce more diacetyl than a sugar did. This may be due to the presence of an unknown sweetener.

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Table 2. Amounts of α-Dicarbonyl Compounds and 4(5)-MI Found in Commercial Soft Drinks

soft drink[a]	glyoxal (μg/mL)	methylglyoxal (μg/mL)	diacetyl (μg/mL)	4(5)-MI (ng/mL)
caramel colored				
IA	16.04 ± 5.13a	26.97 ± 10.08a	7.71 ± 1.01bcd	17.35 ± 2.21b
IB	0.82 ± 0.03c	0.99 ± 0.26c	8.62 ± 0.73abc	8.08 ± 0.56de
IC	0.72 ± 0.11c	1.25 ± 0.76c	7.38 ± 1.54bcd	10.43 ± 0.61cd
IIA	8.72 ± 3.61b	20.09 ± 6.24ab	5.94 ± 1.05cde	28.11 ± 1.92a
IIB	0.71 ± 0.19c	0.77 ± 0.01c	8.33 ± 0.71abc	13.40 ± 4.57c
IIC	0.57 ± 0.05c	0.92 ± 0.30c	9.11 ± 1.43ab	7.22 ± 1.02ef
colorless				
IIIA	13.57 ± 2.03a	19.91 ± 1.98ab	9.91 ± 1.00ab	
IIIB	0.70 ± 0.12c	1.08 ± 0.26c	11.00 ± 2.49a	
IVA	8.63 ± 2.48b	16.24 ± 4.57b	3.48 ± 0.70e	3.52 ± 1.27gh
IVB	0.00 ± 0.00c	0.24 ± 0.07c	4.16 ± 1.32e	1.76 ± 0.74h
VA	8.10 ± 0.82b	20.45 ± 4.40ab	3.51 ± 2.17e	5.51 ± 0.24fg
VC	0.00 ± 0.00c	0.79 ± 0.63c	4.96 ± 2.72de	3.64 ± 0.39gh

[a]A, regular; B, diet; C, zero.

The amount of 4(5)-MI found in commercial product (IA, regular caramel colored) analyzed by LC-MS and GC-NPD was 14.16 ± 4.03 and 17.35 ± 2.21 ng/mL, respectively. These results indicate that the GC-NPD method used in the present study is comparable to the LC-MS method for 4(5)-MI analysis. The amount of 4(5)-MI ranged from 1.76 ± 0.74 ng/mL (IVB, diet colorless) to 28.11 ± 1.92 ng/mL (IIA, regular caramel colored) among the 10 commercial soft drinks analyzed. The amount of 4(5)-MI (ng/mL levels) was considerably lower than those of dicarbonyl compounds (μg/mL levels). When the same caramel colored brands were analyzed for 4(5)-MI in 2011,[6] the amounts found ranged from 300 to 360 ng/mL, suggesting that toxic 4(5)-MI present in commercial soft drinks has been reduced significantly.

The present study demonstrated that glyoxal, methylglyoxal, and diacetyl were formed from a sugar and subsequently produced 4(5)-MI in the Maillard reaction systems. Addition of a sulfite, which has been a common practice to prepare caramel color for soft drinks, reduced formation of 4(5)-MI due to formation of an aldehyde−sulfite adduct. The chemicals investigated in the present study are known to cause tumor formation in experimental animals. In particular, 4(5)-MI has received much attention among regulatory agents as well as consumers. Therefore, finding ways to reduce the formation of these chemicals in beverages is a pressing need. The results of the present study may provide some information to help mitigate the formation of these chemicals.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

GC, gas chromatography; LC-MS, liquid chromatography−mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; 4(5)-MI, 4(5)-methylimidazole; NPD, nitrogen−phosphorus detector; SPE, solid-phase extraction

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