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Formation of Toxic Glyoxal, Methylglyoxal, and Diacetyl Formed in the Headspace of Heated Edible Oils

Qiuhan Wang, Matt Hengel and Takayuki Shibamoto*

Department of Environmental Toxicology, University of California, Davis, CA 95616-8627

***Corresponding author:** T Shibamoto, Department of Environmental Toxicology, University of California at Davis, One Shields Avenue, USA, CA 95616, USA. Email: tshibamoto@ucdavis.edu; Fax: (530) 752-3394; Phone: (530) 752-4523.

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Abstract

Toxic α-dicarbonyl compounds, glyoxal (GL), methylglyoxal (MG), and diacetyl (DA), that formed in the headspace of 50 mL of seven different edible oils heated under simulated cooking conditions, were analyzed. Among the seven edible oils, canola oil generally yielded the highest levels of the three α-dicarbonyl compounds, whereas safflower oil produced the lowest levels. In general, GL was formed from oil samples at the highest levels, ranging from 339 μg (canola) to 19.7 μg (safflower), followed by MG and DA. GL formed in olive and vegetable oils was significantly greater at 200 °C than at 150 °C. The total amounts of α-dicarbonyl compounds recovered ranged from 725 μg (olive oil at 250 °C for 1 h) to 46.3 μg (safflower oil at 150 °C for 0.5 h). The results suggest that these toxic α-dicarbonyl compounds escape into the ambient air during the cooking of lipid-rich foods, and that people are exposed to them.

Keywords: *Edible oils; Deep fat frying; Diacetyl; Glyoxal; Headspace analysis; Methylglyoxal*

Introduction

Edible oils prepared from plants have been used for cooking since ancient times. The ancient Romans first used deep fat frying to prepare Pullum Frontonianum, a chicken dish [1]. Deep fat frying produces desirable flavors and tastes in various fried foods, such as fried chicken, potato chips, and French fries. On the other hand, many studies have demonstrated that edible oils form various toxic low molecular weight carbonyl compounds upon heat treatments [2,3]. Among these carbonyl compounds, α-dicarbonyl compounds, such as glyoxal (GL), methylglyoxal (MG), and diacetyl (DA), reportedly cause various diseases, including cancer, diabetes, and cardiovascular diseases [4,5].

The analysis of low molecular weight carbonyl compounds, including α-dicarbonyl compounds, is extremely difficult because they are highly volatile, reactive, and soluble both in water and in oils. In addition, they are readily polymerized [6]. Moreover, it is almost impossible to isolate them directly from lipids or lipid-rich foods. Therefore, various headspace methods have been developed [7]. Use of the headspace method to investigate formation of vapor phase chemicals from heated edible oils is ideal for assessing the inhalation toxicities of chemicals.

Due to the unique chemical properties of these compounds mentioned above, derivatization is required for the sample preparation [7]. The most commonly used method for trapping vapor-phase chemicals in a headspace is to use impingers containing appropriate derivatizing agents, such as cysteamine and *o*-phenylenediamine. For example, trace levels of volatile aliphatic aldehydes were successfully analyzed in the headspace of heated pork fats [8], food oils [9], and fish flesh [10] using cysteamine derivatives. In addition, α-dicarbonyl compounds formed in the headspace of heated dietary oils [4] and lipid commodities [3] were successfully analyzed using *o*-phenylenediamine derivatives (quinoxalines).

In the present study, GL, MG, and DA formed in the headspace of edible oils heated under simulated cooking conditions were analyzed by a gas chromatograph with a nitrogen phosphorous detector (GC/NPD) after they were derivatized into a corresponding quinoxaline.

Materials and Methods

Chemicals and Reagents

Standard GL, MG, DA, quinoxaline (QX), 2-methylquinoxaline (2-MQX), 2,3-dimethylquinoxaline (2,3-DMQX), *o*-phenylenediamine dihydrochloride, benzothiazole, and sodium hydroxide were purchased from Sigma Aldrich Co. (Milwaukee, WI, USA).

Edible oil Samples

Canola oil, corn oil, vegetable oil (soybean oil), olive oil, peanut oil, grapeseed oil, safflower oil, and blended oil were bought from a local store in Davis, CA, USA.

Determination of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ) for Quinoxalines

A standard ethyl acetate solution (10 mL) containing 100 μg each of QX, 2-MQX, and 2,3-DMQX was prepared. The standard solution was diluted with ethyl acetate in series until an appropriate concentration for LOD and LOQ measurement was reached. The LOD is the concentration giving the GC peak height of the chemical/the highest peak height of the noise (S/N) ratio of 3/1. The LOQ is the concentration giving an S/N ratio of 10/1.

Preparation of a Standard Curve for Quantitative Analysis

For the calibration curves, ethyl acetate solutions of the three quinoxaline derivatives were prepared at seven concentrations (1, 5, 10, 50, 100, 500, 1000 μg/mL). Benzothiazole (200 μg/mL) was spiked as the internal standard prior to GC analysis. The R^2 values ranged from 0.97 to 0.99.

Preparation of an *o*-Phenylenediamine Ethyl Acetate Solution for Synthesis of Quinoxaline Derivatives

In order to prepare a trapping ethyl acetate solution for the impinger, *o*-phenylenediamine dihydrochloride (600 mg) was dissolved in 150 mL of deionized water and the pH was adjusted to 12 with a 2 N sodium hydroxide solution. The chloride salt form of *o*-phenylenediamine dihydrochloride is readily soluble in water but it is not soluble in organic solvents, including ethyl acetate, under neutral conditions. Therefore, a basic aqueous condition (12 pH) was used to release the *o*-phenylenediamine, and then the solution was extracted for free *o*-phenylenediamine with 150 mL ethyl acetate. This extract was used to trap vapor phase GL, MG, and DA formed from heated edible oils. When the headspace of the heated oil samples was analyzed with a GC, a huge peak of unreacted *o*-phenylenediamine appeared in each gas chromatogram, indicating that the trapping ethyl acetate used contained a sufficient amount of the derivatizing agent *o*-phenylenediamine.

Examination of Reaction Yields between α-Dicarbonyl Compounds and o-Phenylenediamine

GL, MG, and DA were spiked (20 μg/mL and 100 μg/mL) to an ethyl acetate solution of *o*-phenylenediamine (150 mL) prepared as described above in a 250 mL capped Erlenmeyer flask. The flask was heated at 40 °C for 40 min in a water bath with a shaker. After the reaction solution was concentrated to approximately 4 mL, 100 μL of benzothiazole (10 mg/mL) was added as a GC internal standard, which was then adjusted to exactly 5 mL with ethyl acetate. The three quinoxaline derivatives were quantitatively analyzed with a GC. The experiments were repeated three times.

Recovery Efficiency of Quinoxaline Derivatives from an Ethyl Acetate Solution

One μg each of standard QX, 2-MQX, and 2,3-DMQX were dissolved into 150 mL ethyl acetate. The solution was concentrated to a little more than 5 mL by a rotary evaporator and its volume was reduced to exactly 5 mL by purging with a pure nitrogen stream. The concentrated ethyl acetate solution was analyzed for the three quinoxaline derivatives by a GC. The experiment was repeated six times.

Analysis of GL, MG and DA Formed in the Headspace of Heated Edible Oils

Figure 1 shows a schematic of the apparatus used to trap QX, 2-MQX, and 2,3-DMQX formed in the headspace of heated edible oils. An oil sample (50 mL) was placed in a 2-neck, round-bottom flask connected to 2 tandem impingers containing 150 mL each of the ethyl acetate solution prepared as described above. The flask was heated and when the sample temperature reached 150 °C, 200 °C or 250 °C, the heated sample was purged by an air stream (30 mL/min) to introduce the vapor phase GL, MG, and DA formed in the headspace of the heated edible oils into the impingers for 0.5 h or 1 h. Following the headspace collection, the trapping solution was transferred to a capped flask and was shaken in a water bath at 25 °C for 40 min. A rotary evaporator was used to concentrate the extract held at 40 °C using a water bath. One hundred μL of benzothiazole (10 mg/mL) was added as a GC internal standard, after which the extract was adjusted to exactly 5 mL with ethyl acetate. The concentrated sample was analyzed for GL, MG, and DA as a corresponding quinoxaline derivative by a GC/NPD. Blank unheated samples of each oil were prepared following the same procedures as heated samples.

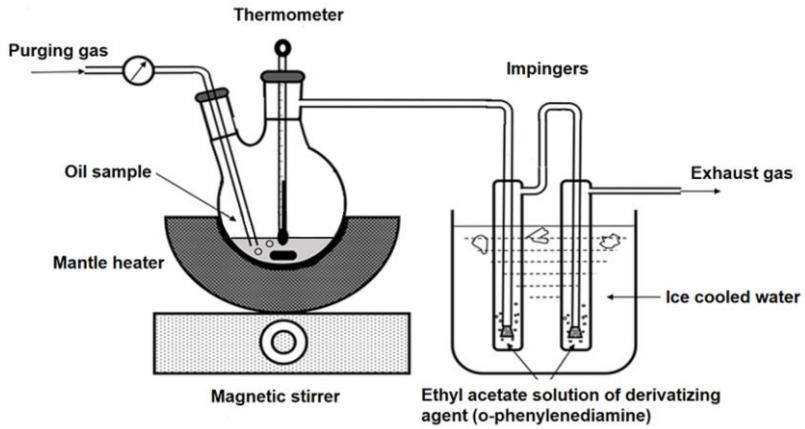

Figure 1: The apparatus used to trap α-dicarbonyl compounds formed in the headspace of heated edible oils.

Instrumental

An Agilent Model HP 6890 series GC equipped with a 30 m × 0.25 mm i.d. x 0.25 μm DB-WAX fused silica capillary column and an NPD was used for the quantitative analysis of the quinoxalines. The injector and detector temperatures were 260 °C and 300 °C, respectively. The split ratio was 70:1. The oven temperature was held at 70 °C for 2 min, then programmed 170 °C at 5 °C/min, then held for 8 min; to 180 °C at 10 °C/min, then held for 7 min. The helium carrier gas flow rate was 2.3 mL/min.

Identification of QX, 2-MQX, and 2,3-DMQX in each sample was performed using an HP Model 6890 GC interfaced to a 5973 MSD (GC/MS) with the same GC conditions as described above. The quinoxaline derivatives were identified by the mass spectral fragmentation pattern and GC retention time of each standard compound.

Statistical Analysis

The results of the present study were averaged and the comparisons between experimental groups were drawn through a JMP program. After the ANOVA analysis, the level of significance was computed using the Tukey HSD test at α = 0.05.

Results and Discussion

The purpose of the present study is to determine the amounts of GL, MG, and DA formed in the headspace of heated edible oils, not in the oil itself. These toxic α-dicarbonyl compounds are expected to escape into the ambient air during cooking and subsequently to be inhaled by people. It is important to know the possible amounts of those chemicals produced in a headspace in order to assess their inhalation toxicities.

LOD, LOQ and Recovery Efficiencies

The LOD of QX was 0.08 ng, that of 2-MQX was 0.12 ng and that of 2,3-DMQX was 0.05 ng. The LOQ of QX was 0.28 ng, that of 2-MQX was 0.40 ng and that of 2,3-DMQX was 0.15 ng. The apparatus initially had two impingers in series, but it was found that no target compounds were recovered from the second impinger. Therefore, the single impinger system was used for all later experiments.

The Reaction Yields of Quinoxaline Derivatives and Their Recovery Efficiencies from Ethyl Acetate Solutions

The reaction yields of QX were 118.5 ± 1.4% and 75.4 ± 11.8% at the levels of 20 μg/mL and 100 μg/mL, respectively; 2-MQX yields were 78.1 ± 5.4% and 77.7 ± 1.9% at the of levels of 20 μg/mL and 100 μg/mL, respectively; 2,3-DMQX yields were 62.7 ± 8.2% and 75.6 ± 4.5%, at the levels of 20 μg/mL and 100 μg/mL, respectively. Values are the mean ± RSD (n = 3).

The recovery efficiency of quinoxalines from an ethyl acetate solution was 81.5 ± 1.40% for QX, 94.8 ± 4.60% for 2-MQX, and 105 ± 3.80% for 2,3-DMQX. The values are mean ± SD (n = 6). The results indicate that the derivatives were satisfactorily recovered from the impingers.

The Levels of GL, MG, and DA Recovered from the Headspace of Seven Edible Oils Heated under Different Conditions

Figure 2 shows the levels of GL, MG, and DA recovered from the headspaces of 50 mL each of seven edible oils heated for 0.5 h at 150 °C (**A**), 200 °C (**B**), and 250 °C (**C**). The values are mean ± SD (n = 3). When the oil samples were heated at 150 °C (**A**), GL was recovered in the highest levels, ranging from 63.6 ± 10.5 μg (grapeseed) to 19.7 ± 0.56 μg (safflower). MG recovered ranged from 36.6 ± 6.85 μg (canola) to less than LOQ (peanut and safflower). DA recovered ranged from 32.0 ± 2.59 μg (peanut) to less than LOQ (canola and vegetable).

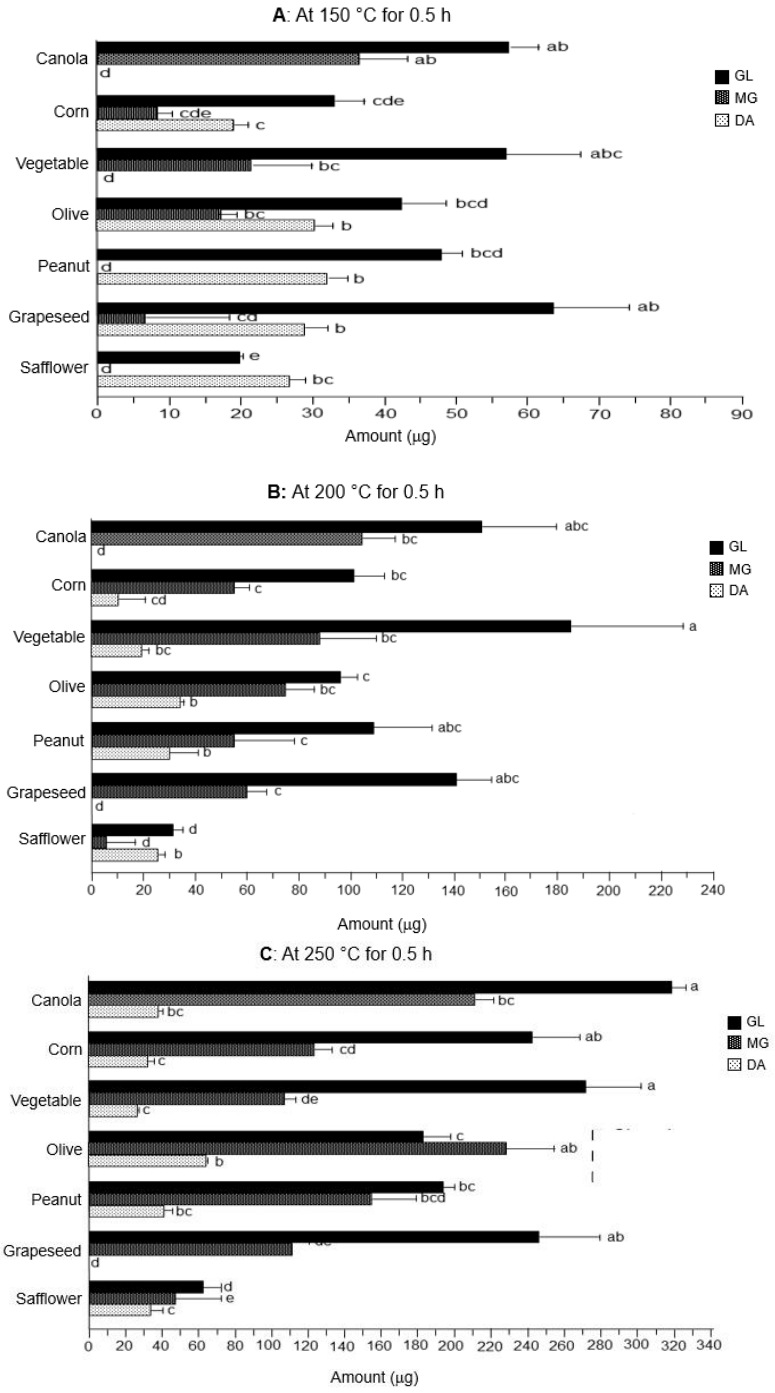

Figure 2: The levels of GL, MG, and DA recovered from the headspaces of 50 mL each of seven edible oils heated for 0.5 h at 150 °C (A), 200 °C (B), and 250 °C (C).

When the oil samples were heated at 200 °C (**B**), GL was recovered in the greatest amounts, ranging from 185 ± 43.2 μg (vegetable) to 31.1 ± 3.88 μg (safflower). MG recovered ranged from 104 ± 12.8 μg (canola) to 3.85 ± 4.57 μg (safflower). DA was recovered at the lowest levels among the three compounds except in the case of safflower oil, ranging from 34 ± 1.65 μg (olive) to less than LOQ (canola and grapeseed).

When the oil samples were heated at 250 °C (**C**), GL was recovered in the greatest amounts except in the case of olive oil, ranging from 317 ± 7.38 μg (canola) to 62.0 ± 9.73 μg (safflower). MG recovered ranged from 228 ± 26.4 μg (olive), which was higher than that of GL, to 46.9 ± 25.0 μg (safflower). It is interesting that more MG was recovered than GL from olive oil. DA recovered ranged from 64.9 ± 0.58 μg (olive) to less than LOQ (grapeseed).

Figure 3 shows the levels of GL, MG, and DA recovered from the headspaces of 50 mL each of seven edible oils heated for 1 h at 150 °C (**A**), 200 °C (**B**), and 250 °C (**C**). The values are mean ± SD (n = 3). When oil samples were heated at 150 °C (**A**), GL was recovered in the greatest amounts from all seven oils except safflower, with yields ranging from 223 ±

36.3 µg (canola) to 23.3 ± 1.56 µg (safflower). MG recovered ranged from 139 ± 36.3 µg (canola) to less than LOQ (safflower). DA recovered ranged from 48.7 ± 3.11 µg (peanut) to 25.4 ± 2.18 µg (corn). In the case of safflower, DA (37.5 ± 5.59 µg) was recovered in the greatest amount of the three α-dicarbonyl compounds.

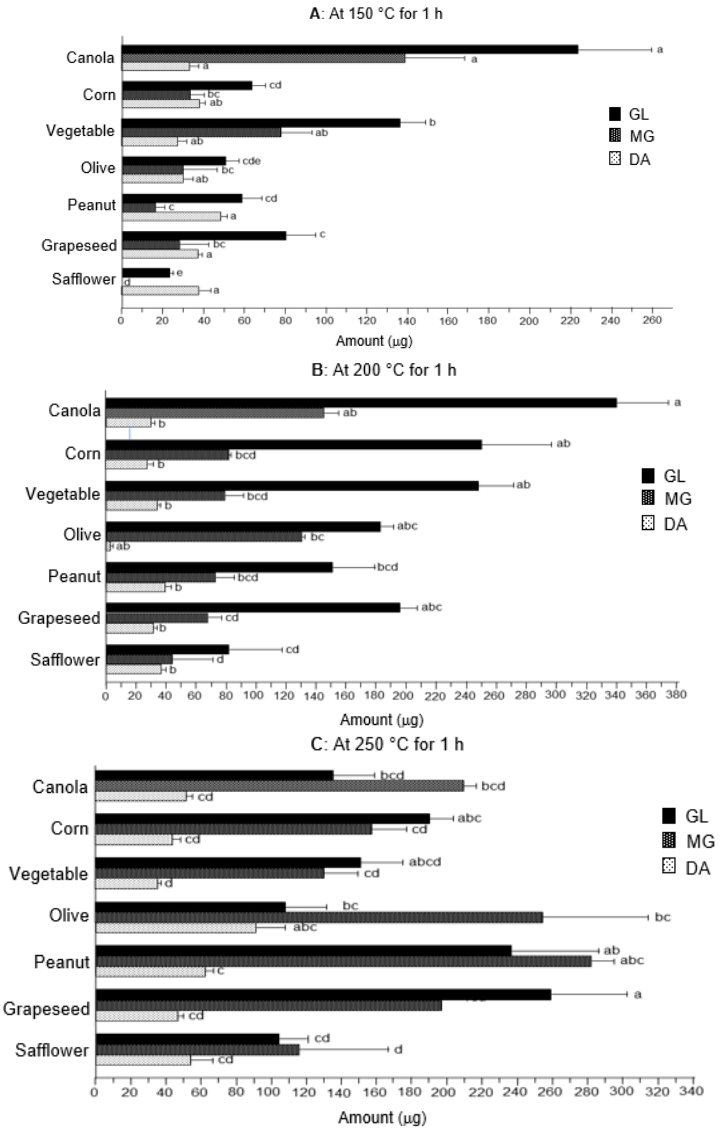

Figure 3: The levels of GL, MG, and DA recovered from the headspaces of 50 mL each of seven edible oils heated at for 1 h at 150 °C (A), 200 °C (B), and 250 °C (C).

When the oil samples were heated at 200 °C (**B**), GL was recovered in the greatest amounts from all seven edible oils. Canola produced the highest level of GL (339 ± 34.1 µg), followed by corn (249 ± 46.8 µg), and grapeseed oil (81.3 ± 34.5 µg). Safflower yielded the least (81.3 ± 34.5 µg). MG recovered ranged from 144 ± 9.30 µg (canola) to 44.2 ± 27.2 µg (safflower). DA recovered ranged from 52.8 ± 1.26 µg (olive) to 27.3 ± 3.57 µg (corn).

When the oil samples were heated at 250 °C (**C**), which was the most vigorous heating condition used in the present study, MG was recovered in the greatest amounts from canola, olive, peanut, and safflower oils, ranging from 281 ± 12.3 µg (peanut) to 115 ± 51.1 µg (safflower), of the three µ-dicarbonyl compounds. GL recovered ranged from 258 ± 43.1 µg (grapeseed) to 103 ± 16.5 µg (safflower). DA recovered ranged from 110 ± 14.6 µg (olive) to 35.5 ± 1.79 µg (vegetable). The main difference observed in the results from this sample is the formation of relatively higher levels of MG than GL, which generally formed in the greatest amounts in the oil samples heated to lower temperatures and for shorter times.

When heating time was increased from 0.5 h to 1 h, GL increased significantly in the cases of canola, corn, and vegetable oils, whereas it increased only slightly from the remaining four oils. It is obvious that extension of heating time increased the oxidative degradation of the oils.

The GL in safflower oil increased noticeably at 250 °C but showed no change at lower temperatures. When the edible oils were heated for 0.5 h at all three temperatures, the amount of MG in canola, corn, olive, and peanut oils increased considerably as temperatures increased, but there were only slight increases in the case of grapeseed and vegetable oils from 150 °C to 200 °C. When the heating

time increased to 1 h, the amount of MG in canola and vegetable oils did not change significantly.

GL promoted rat glandular stomach carcinogenesis [11]. The mutagenicity of GL was reported in the early 1990s [12]. The previous study indicated that GL might be a potent inducer for diabetes-associated vascular endothelial cell injury [13]. Also, dose-dependent toxicity of GL was observed in isolated rat liver mitochondria [14]. MG was found in brewed coffee at a level of 25 μg/g [15]. Later, MG was reported to be a cytotoxic factor in coffee and drinking an excess of any type of coffee may be a health risk [16]. MG was shown to have biological implications, such as cancer formation [17,18]. An experimental animal study demonstrated that MG reduced memory retention when it was administered to mice [19]. Another recent study with experimental animals demonstrated that MG possesses colorectal cancer-promoting properties in murine models [20].

The amount of DA only increased significantly when the temperature was raised from 200 °C to 250 °C in canola and olive oils heated for 0.5 h. DA formation levels did not change significantly in corn, peanut, and safflower oil. Interestingly, the amount of DA in grapeseed oil decreased considerably as the temperature rose from 150 °C to 200 °C; that is the opposite from the results observed in the case of vegetable oil. When the heating time increased to 1 h, the amount of DA in safflower and vegetable oils did not change, whereas all other oils showed a significant increase of DA levels when the temperature rose from 200 °C to 250 °C, increases that were not observed in the lower temperature ranges.

DA has been used for giving a butter flavor to popcorn but is known to cause respiratory diseases among workers in popcorn factories because it vaporizes during food processing and escapes into ambient air [2]. On the other hand, DA concentrations in mainstream cigarette smoke ranged from 250 to 361 ppm for all tobacco products, which suggests that DA exposure from cigarette smoke far exceeds occupational exposures for most food/flavoring workers who smoke [21]. Nonetheless, a recent study reported that exposure to DA caused respiratory tract damage in humans and experimental animals.

Generally, it was observed that the formation of these compounds was proportional to time and temperature, suggesting that the oil degradation occurs more at the higher and longer conditions. On the other hand, when canola, corn, and vegetable oils were heated at 250 °C for 1 h, GL formation reduced significantly from its formation at the same temperature for 0.5 h. This may be due to the progress of the polymerization of GL after 0.5 h heat treatment at a high temperature. In addition, the GL may have been trapped with some other constituents during cooling.

Table 1 shows the total amounts of α-dicarbonyl compounds recovered from edible oils heated under various conditions (prepared from the data in Figures 2 and 3) and the polyunsaturated fatty acid compositions of each oil sample [22]. The total amounts of α-dicarbonyl compounds recovered from the headspaces of the seven edible oils ranged from 726 μg (olive oil at 250 °C for 1 h) to 46.3 μg (safflower oil at 150 °C for 0.5 h). When the seven edible oils were heated at 150 °C for 1 h, at 200 °C for 0.5 h and 1 h, and at 250 °C for 0.5 h, canola oil produced the highest level of total α-dicarbonyl compounds. On the other hand, when the seven edible oils were heated at 250 °C for 1 h, olive oil (726 μg), peanut oil (578 μg) and grapeseed oil (500 μg) produced more of these compounds than canola oil (395 μg) did, suggesting that some different mechanism is obtained under more vigorous heating conditions.

Heating Conditions							
	150 °C		**200 °C**		**250 °C**		
Oil Samples	0.5 h	1 h	0.5 h	1 h	0.5 h	1 h	PUFA (%)
Canola	94.1	395	256	513	566	395	92
Corn	60.4	122	162	358	397	390	84
Vegetable	78.4	242	292	359	403	315	81
Olive	90.2	111	205	365	427	726	86
Peanut	80.0	124	194	261	387	578	79
Grapeseed	99.1	146	201	293	356	500	75
Safflower	46.3	60.8	60.2	162	143	272	89

Table 1: Total of α-Dicarbonyl Compounds (Glyoxal, Methyl Glyoxal, and Diacetyl) Formed in the Headspace from 50 mL Each of Edible Oils under Various Heating Conditions and Compositions of PUFA (Polyunsaturated Fatty Acid) of Each Edible Oil. Values are in μg.

Generally, canola oil yielded the highest levels of the three α-dicarbonyl compounds, whereas safflower produced the lowest levels of them in a headspace. Total amounts of α-dicarbonyl compounds recovered from the edible oils were higher after 1 h heating time than 0.5 h heating time when they were heated at 150 °C and 200 °C. On the other hand, when they were heated at 250 °C for 0.5 h, canola, corn, and vegetable oils formed higher amounts of α-dicarbonyl compounds than when they were heated for 1 h at same temperature, indicating that the heating time does not have an important role for formation of α-dicarbonyl compounds at a high temperature.

As mentioned above, it is extremely difficult to construct a simple rule for the formation of GL, MG, and DA from different oils upon heat treatment. However, roughly, it appears that the more unsaturated fatty acids there are in the composition of an edible oil the more α-dicarbonyl compound formation was observed. For example, canola oil, which is composed of 92% unsaturated fatty acids, in all but one case formed the highest levels of total α-dicarbonyl compounds. Only in the case when it was heated at 150 °C for 0.5 h, grapeseed oil, which is composed of 75% unsaturated fatty acids, formed the highest level (99.1 μg) followed by canola oil (94.1 μg).

There have been many reports on the formation mechanisms of lipid peroxidation products, including low molecular weight α-dicarbonyl compounds. These mechanisms have been well established as auto-oxidation of lipids, which occurs under mild conditions [23,24]. However, when exposure to a high energy source, such as high temperatures or UV irradiation, is involved in the lipid peroxidation process, many radicals, such as •H, •CH_3, and •C=O, form at the early stage of oxidation and subsequently these radicals combine with each other to produce carbonyl compounds, including GL, MG, and DA [3,25]. Therefore, the amounts of these α-dicarbonyl compounds may depend on the amounts of these radical formations.

There have been many toxicological studies on these α-dicarbonyl compounds. However, in most studies, these chemicals were administered orally to experimental animals. There is virtually no report on the inhalation toxicities of α-dicarbonyl compounds, including GL, MG, and DA. Because volatile vapor-phase α-dicarbonyl compounds form in the headspace of heated edible oils and subsequently people inhale them during cooking or food processing [3], studies on inhalation toxicity of these α-dicarbonyl compounds are in order. Therefore, it is important to determine the amount of these volatile vapor-phase α-dicarbonyl compounds escaping into the ambient air during cooking or food processing in order to assess their safety levels.

Conclusion

There is no clear evidence of adverse effects, including cancer formation, to people caused by these vapor phase α-dicarbonyl compounds even though they have been shown to be a possible carcinogen in animal studies. In addition, there are no specific studies on the inhalation toxicities of these α-dicarbonyl compounds. The present study demonstrates the formation of toxic GL, MG, and DA in the headspace of edible oils heated under simulated cooking conditions. The amounts of these three total α-dicarbonyl compounds formed in the headspace of seven edible oils varied, but they are not negligible. For example, when 50 mL of canola oil is used for deep-fat frying at 250 °C for 0.5 h, 566 μg of total GL, MG and DA escapes into the ambient air (calculated using the results of the present study). Analysis of the vapor phase α-dicarbonyl compounds is one promising avenue through which to study the inhalation toxicities of these chemicals.

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