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Investigation of Toxic α-Dicarbonyl Compounds Formed in the Headspace of Various Heated Cooking Oils

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ABSTRACT

Glyoxal, methylglyoxal, and diacetyl formed in the headspace of heated oil samples were analyzed as corresponding quinoxaline derivatives. Formation of these compounds increased in the gutter oil as the number of heating repetitions increased. In particular, methylglyoxal increased from 198.43 ± 92.9 µg to 432.16 ± 161.86 µg. The total phenolic compounds in four extra virgin olive oils ranged from 231.01 ± 16.75 µg/g to 492.78 ± 39.34 µg/g (Olio Nuovo olive oil). The carbonyl compounds formed from the Olio Nuovo oil ranged from 52.76 ± 11.26 µg (glyoxal) at 150°C for 0.5 h to 801.22 ± 147.88 µg (diacetyl) at 300°C for 1 h. Addition of cysteine reduced the formation of all three α-dicarbonyl compounds significantly, suggesting that cysteine forms adducts with carbonyl compounds. Standard antioxidant BHT reduced formation of all three compounds at lower heating temperatures, whereas standard antioxidant vitamin E increased them considerably at higher temperatures, suggesting that vitamin E degrades into carbonyl compounds.

Introduction

Low molecular weight carbonyl compounds, such as glyoxal, methylglyoxal and diacetyl, have received much attention as chemicals that have adverse effects on human health [1]. Among them, glyoxal has shown tumor-promoting activity in rat glandular stomach carcinogenesis [2]. Methylglyoxal reportedly plays an important role in the occurrence of cancer and diabetes [3]. Diacetyl caused respiratory toxicity among workers in popcorn plants, but has been used as a flavoring in butter, popcorn, caramel, coffee, and cream soda [4].

These carbonyl compounds form mainly from lipid-rich foods, like beef fat, butter, and cooking oils upon oxidation [4]. Preparation of fast-foods, such as fried chicken, French fries, and potato chips, involves deep-fat frying at high temperatures for a prolonged time and subsequently significant amounts of carbonyl compounds are formed. Consequently, individuals who are exposed to the deep-fat frying vapors either at work or at home are potentially at risk of adverse health effects.

In China, so-called gutter oils have been heavily used in the food services and catering industries. Gutter oils are prepared from waste oils recycled from restaurants, cafeterias, sewage drains, and slaughterhouse waste after intensive refining processes [5]. Large amounts of gutter oils (10% of the edible oils consumed in China) are produced and re-used in restaurants in China [5]. Clearly, gutter oils are expected to contain high levels of toxic carbonyl compounds because they are more oxidized than fresh cooking oils. Further, chronic consumption of gutter oils may cause some adverse health effects because the oils contain heavy metals, bacteria, and carcinogens in addition to toxic low molecular weight carbonyl compounds [6].

Constant and consistent inhalation of toxic carbonyl compounds, even in trace amounts, may cause some adverse effects to human health. Therefore, there is a pressing need to obtain accurate data on the formation of these toxic carbonyl compounds from cooking oils to establish safety guidelines. However, analysis of low molecular

weight carbonyl compounds in a food matrix is extremely difficult because they are highly volatile and reactive, miscible in water and oil, and readily form adducts with food components [1]. Recently, we developed a new method for the analysis of toxic α-dicarbonyl compounds, including glyoxal, methylglyoxal, and diacetyl, formed in the headspace of heated cooking oils [7,8].

In the present study, the formation of toxic glyoxal, methylglyoxal, and diacetyl in the headspace of various cooking oils heated under different conditions was investigated using the above-mentioned newly developed analytical method.

Materials and Methods
Chemicals and cooking oil samples
Standard glyoxal, methylglyoxal, diacetyl, quinoxaline, 2-methylquinoxaline, 2,3-dimethylquinoxaline, benzothiazole, o-phenylenediamine dihydrochloride, and sodium hydroxide were purchased from Sigma Aldrich Co. (Milwaukee, WI, USA). Folin-Ciocalteu reagent (2 N), standard gallic acid, and sodium carbonate were also bought from Sigma Aldrich Co. (Milwaukee, WI, USA). Stock solution of gallic acid was prepared in methanol/water mix (60:40 vol/vol) at 10 mg/mL and stored at 4°C.

Safflower oil, canola oil, olive oil, corn oil, vegetable oil, grapeseed oil, and blend oils (olive, canola, and grapeseed oils) were purchased from a local store in Davis, CA. Simulated gutter oils were prepared with a mixture of previously heated (at 250°C for 1 hour) above-mentioned cooking oils. The Olio Nuovo model oil was purchased from McEVOY Ranch (Petaluma, CA, USA), which was the freshest (harvested in Autumn 2017) unfiltered extra virgin olive oil bottled immediately after the first cold press and centrifugal separation.

Sample preparation and analysis
The methods used in the present study were developed in our lab; for details, see Wang et al. [8]. Therefore, they are just briefly described here.

Glyoxal, methylglyoxal, and diacetyl formed in the headspace of cooking oils heated under different conditions were derivatized into corresponding quinoxalines with o-phenylenediamine. The quinoxaline derivatives were recovered and analyzed using the methods reported in the previous study [8]. Briefly, a cooking oil (50 mL) was added to a 2-neck round bottom flask connected to two tandem impingers containing 150 mL each of the ethyl acetate solution with derivatizing reagent, o-phenylenediamine. The cooking oil was heated at 150°C, 200°C, or 250°C for 0.5 h or 1h. The headspace of the flask was purged by an air stream (30 mL/min) into the impingers during heating. The combined trapping solutions from two impingers was concentrated to exactly 5 mL using a rotary evaporator, and then benzothiazole (10 mg/mL) was added as a GC internal standard. The corresponding quinoxaline derivatives (quinoxaline, 2-methylquinoxaline, and 2,3-dimethylquinoxaline) formed from glyoxal, methylglyoxal, and diacetyl were analyzed by a gas chromatograph equipped with a nitrogen phosphorus detector (GC-NPD). A non-heated oil was used as a blank sample. The experiment was repeated three times.

Instrumental
An Agilent Model HP 6890 series GC equipped with a DB-WAX fused silica capillary column (30 m × 0.25 mm i.d. x 0.25 μm) and a nitrogen phosphorus detector (NPD) was used for analysis of samples. The injector was operated at 260°C with 70:1 split ratio.

The GC oven temperature was held at 70°C for 2 min, and then programed to 180°C at 5°C/min and held for 7 min. The carrier gas (helium) flow rate was 2.3 mL/min. The detector temperature was held at 300°C.

HP Model 6890 GC connected to a 5973 MSD (GC-MS) and operated under the same GC conditions were used for the identification of quinoxaline, 2-methylquinoxaline, and 2,3-dimethylquinoxaline in each sample. The mass spectral fragmentation pattern and GC retention time of each compound were used to identify each quinoxaline derivative.

Standard curve preparation for GC quantitative analysis
The calibration curves for GC quantitative analysis for the three quinoxaline derivatives were prepared at six concentrations (1, 5, 10, 50, 100, 500 μg/mL) using corresponding standard quinoxaline solutions with benzothiazole (100 μg/mL) as an internal standard in ethyl acetate solution [9]. The R^2 values of calibration curves were > 0.99.

Study of simulated gutter oils
Simulated gutter oils were prepared from a mixture of selected cooking oils. A simulated gutter oil was heated once, twice, and three times at 250°C for 1 h each to examine the repeated heating effect on the formation of toxic α-dicarbonyl compounds. Before repeat heating, the heated oils were left to stand until they cooled down to 25°C and then heated to 250°C. A fresh olive oil (50 mL) alone was treated with the same procedures as the gutter oils. The three α-dicarbonyl compounds formed in the headspace of the samples were analyzed using the method described above. The experiment was repeated three times.

Measurement of total phenolic content
In the present study, four extra virgin olive oils were used. They were 1). EVOO 1: Made in Spain using Spanish olives, 2). EVOO 2: Made in California using Arbequinoa olives, 3). EVOO 3: Made in California using Spanish olives, 4). EVOO 4: Made in California using Frantoio, Leccino, Pendolino, Moraiolo, Maurino, Coratina, and Leccio Del Corno olives, which is also called Olio Nuovo extra virgin olive oil.

Measurement of total phenolic content (gallic acid equivalent, GAE) in oil samples was performed by a previously published method [10]. Briefly, an oil sample (2.5 mL) was dissolved in hexane (5 mL). The phenolic compounds were extracted with 3 mL methanol/water (60:40 vol/vol) solution by vortex for 2 min, and then the extract was centrifuged at 3500 rpm for 10 min. The upper layer was re-extracted with 3 mL of the same methanol/water solution and the extract was combined with the lower layer. An aliquot (0.2 mL) of extract was diluted with water to 2.5 mL.

Then Folin-Ciocalteu reagent (0.25 mL) was added. After 0.5 mL of sodium carbonate solution (35%, wt/vol) was added, it was diluted to 5 mL with water. Blank samples were prepared the same way without the oil samples. After incubation at room temperature for 90 min, the absorbance of the samples was measured at wavelengths of 290 nm, 300 nm, 310 nm, 550 nm, and 760 nm. The maximum absorbance occurred near 300 nm. The experiment was run in triplicates.

Investigation of the role of antioxidants in α-dicarbonyl compounds formation

Commercial cooking oils contain some naturally occurring antioxidants, such as phenolic compounds, and artificially added antioxidants, such as butylated hydroxytoluene (BHT) and vitamin E. To investigate the effect of antioxidants on the formation of α-dicarbonyl compounds, 2 g each of standard BHT and vitamin E were added to the Olio Nuovo extra virgin olive oil (50 mL) and heated at 150°C, 200°C, 250°C for 0.5 h. A blank was prepared without the addition of BHT or vitamin E. The glyoxal, methylglyoxal, and diacetyl formed in the headspace of the samples were analyzed as described above. The experiment was repeated three times.

Food matrix study

Two grams each of standard cysteine, *D*-glucose, and starch was added to Olio Nuovo extra virgin olive oil (50 mL), which was heated at 250°C for 0.5 h. Olive oil alone was also heated under the same conditions and the results were used as a blank sample. The glyoxal, methylglyoxal, and diacetyl formed in the headspace of the samples was analyzed as described above. The experiment was repeated three times.

Statistical analysis

An ANOVA analysis performed in JMP using the Tukey HSD test ($\alpha = 0.05$) was used for statistical analysis of the results.

Results and Discussion

As mentioned above, the limit of detection (LOD) and the limit of quantitation (LOQ) for the three quinoxaline derivatives were the same as the values reported previously [8].

Gutter oil studies

Figure 1 shows the results of gutter oil studies. A gutter oil and an olive oil were treated with three heating repetitions and with four heating repetitions, respectively. Amounts of the three α-dicarbonyl compounds recovered increased steadily when the number of heating repetitions increased. When the number of repetitions increased from one (Gutter 1) to three times (Gutter 3), the amounts of α-dicarbonyl compounds increased from 123.51 ± 20.32 µg to 181 ± 62.92 µg for glyoxal, from 198.43 ± 92.9 µg to 432.16 ± 161.86 µg for methylglyoxal, and from 54.47 ± 28.42 µg to 79.09 ± 20.18 µg for diacetyl.

In the case of the olive oils, it is interesting that the formation of glyoxal reduced steadily from 107.58 ± 23.74 µg (Olive 1) to 80.94 ± 3.96 µg (Olive 4), when the heating repetition increased. Glyoxal may have polymerized and/or formed adducts with other oil constituents during the trapping processes because of its high reactivity. On the other hand, methylglyoxal (from 253.62 ± 60.44 µg to 361.2 ± 91.31 µg) and diacetyl (from 91.24 ± 16.44 µg to 112.17 ± 5.51 µg) increased consistently with the increase of repetitions.
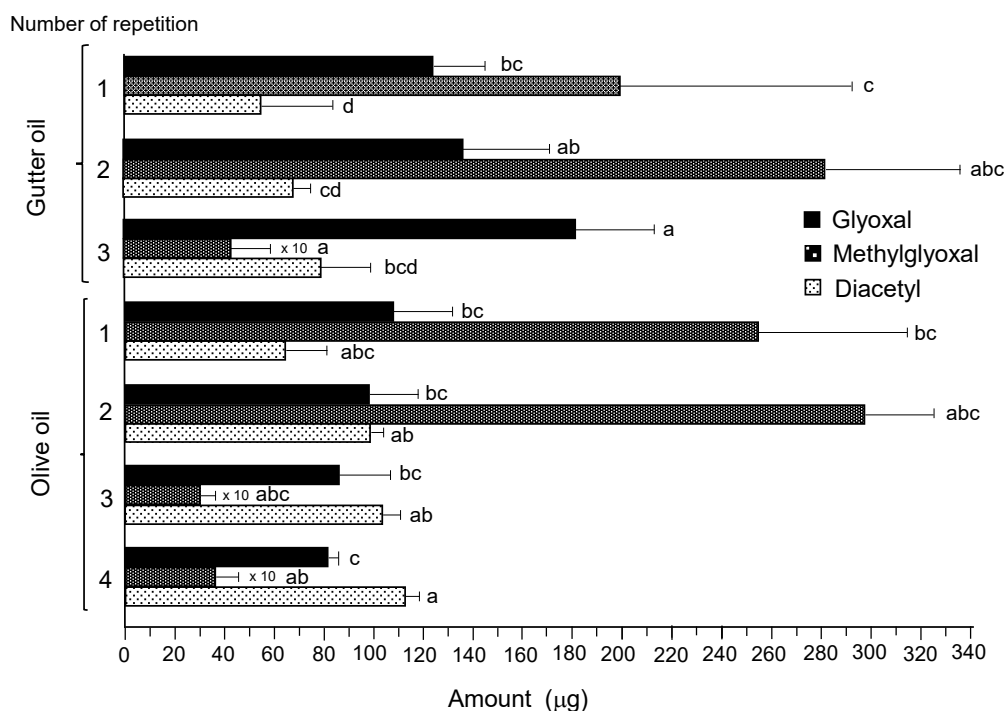

Figure 1: The results of the simulated gutter oil study. The values are mean ± standard deviation (n = 3).

The overall results of the present study suggest that just one kind of oil does not play an important role in the formation of three α-dicarbonyl compounds in gutter oil. Moreover, commercial gutter oils consist of extremely complex matrices because they are made from various waste materials, followed by many intensive preparation processes, including filtration, boiling, refining, and the removal of some adulterants [5]. Therefore, further studies are required to assess the safety of gutter oils.

Total phenolic contents in model oils

Figure 2 shows the phenolic contents of the four model oils. The phenolic contents ranged from 231.01 ± 16.75 µg/g (EVOO 2) to 492.78 ± 39.34 µg/g (EVOO 4). Three oils (EVOO 1, 2, and 3) exhibited similar levels of phenolic contents. On the other hand, EVOO 4 showed a considerably higher level. The levels found in the present study were lower than those in plant oils reported previously [11] except for the level found in EVOO 4. Therefore, this oil was used for further investigation.

The results of the Olio Nuovo model oil study

Figure 3 shows the results of the three α-dicarbonyl compounds analysis from Olio Nuovo oil (EVOO 4) heated at 150°C, 200°C, 250°C, or 300°C for 0.5 h or 1 h. Generally, the amount of three

α-dicarbonyl compounds increased when heating temperature and time increased from 150°C to 300°C and from 0.5 h to 1 h, respectively.

The levels of glyoxal ranged from 52.76 ± 11.26 µg (at 150°C for 0.5 h) to 175.42 ± 9.79 µg (at 250°C for 0.5 h). On the other hand, when the oil was heated for 1 h, the highest level of glyoxal was observed at 200°C. No specific trend was exhibited in the case of glyoxal formation.

Methylglyoxal formed a continuous increasing trend as temperature and time rose. In sum, the level of methylglyoxal increased from not detected (at 150°C for 0.5 h) to 404.03 ± 200.74 µg (at 300°C for 1 h). It is interesting that no detectable level of diacetyl was observed in the oil heated at 150°C, 200°C, and 250°C for 0.5 h; and 150°C and 200°C for 1 h. The formation of diacetyl appeared suddenly at higher temperatures. The level of diacetyl formed in the oil heated at 300°C for 1 h was 801.22 ± 147.88 µg, which was the highest level found in all samples.

Antioxidant effects

Figure 4 shows the antioxidant effects on the three α-dicarbonyl compounds formed from heated oils under various conditions.

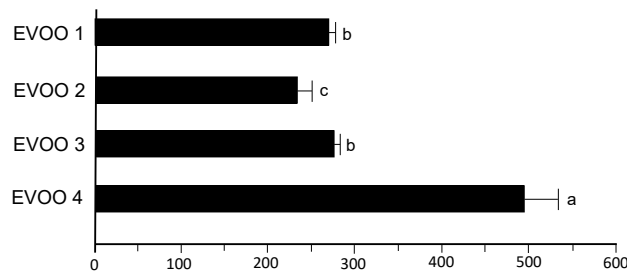**Figure 2:** The phenolic contents of four extra virgin olive oils. The values are mean ± standard deviation (n = 3).
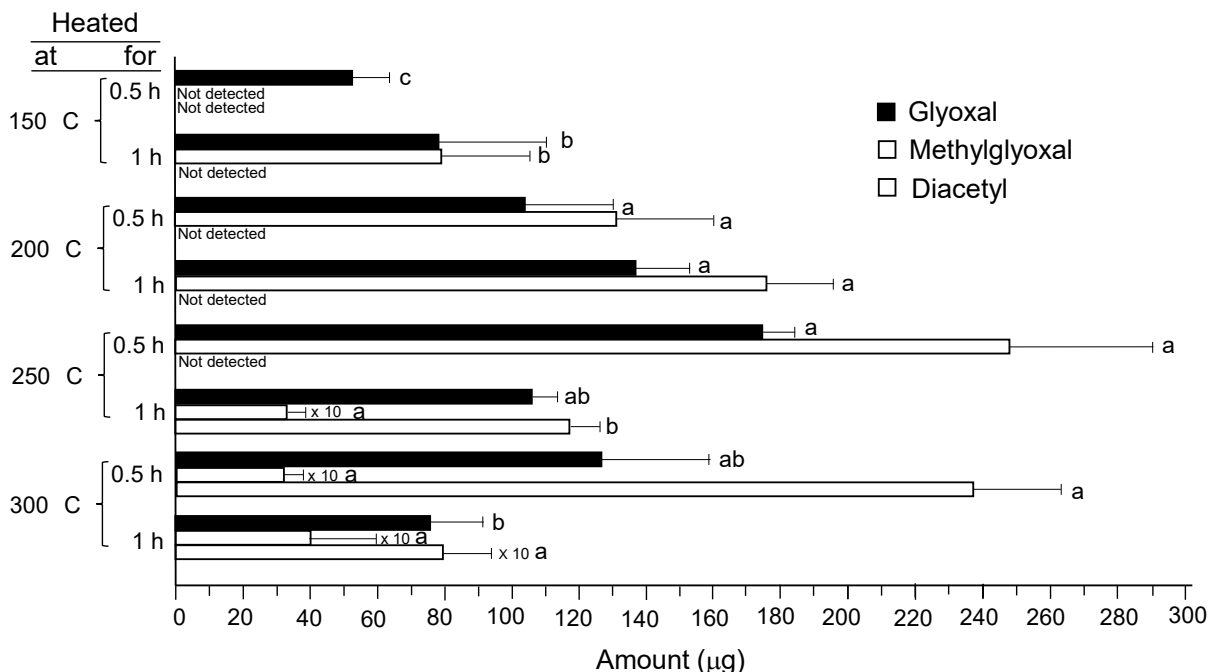

Figure 3: The results of three α-dicarbonyl compounds analysis from Olio Nuovo extra virgin olive oil. The values are mean ± standard deviation (n = 3).
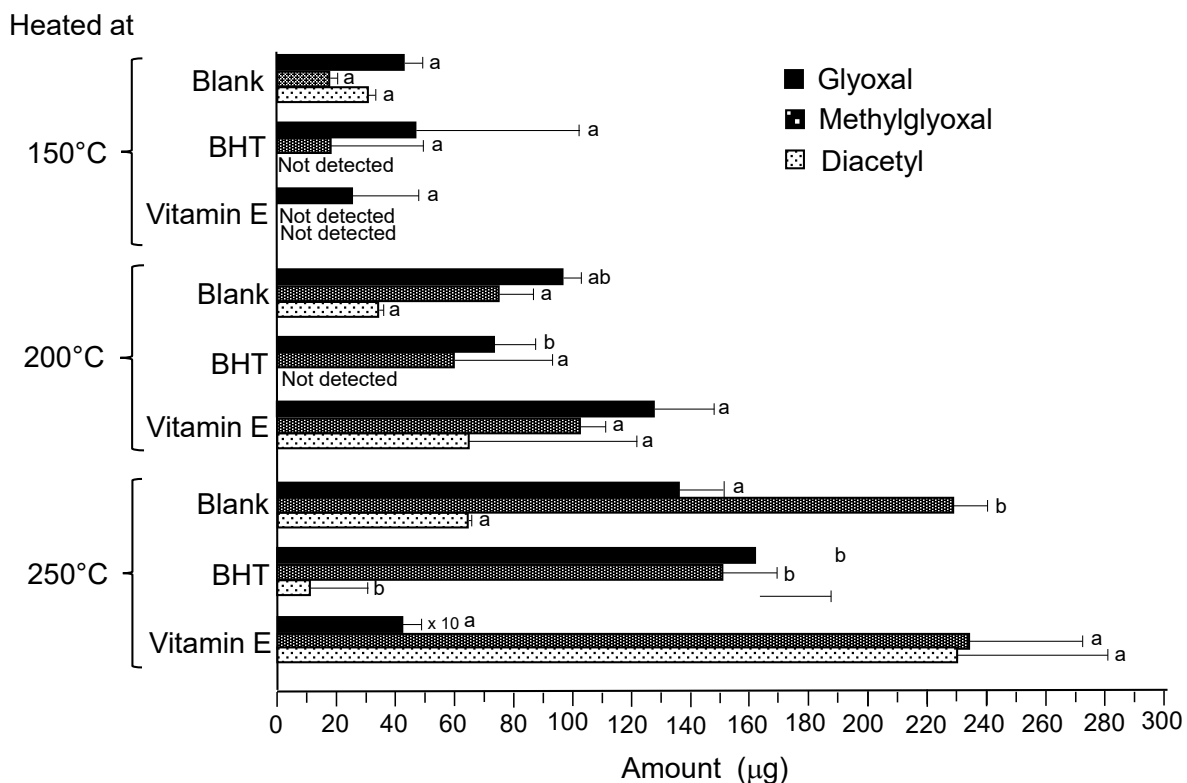
Figure 4: The antioxidant effects toward formation of three α-dicarbonyl compounds from the heated Olio Nuovo extra virgin olive oils. The values are mean ± standard deviation (n = 3).
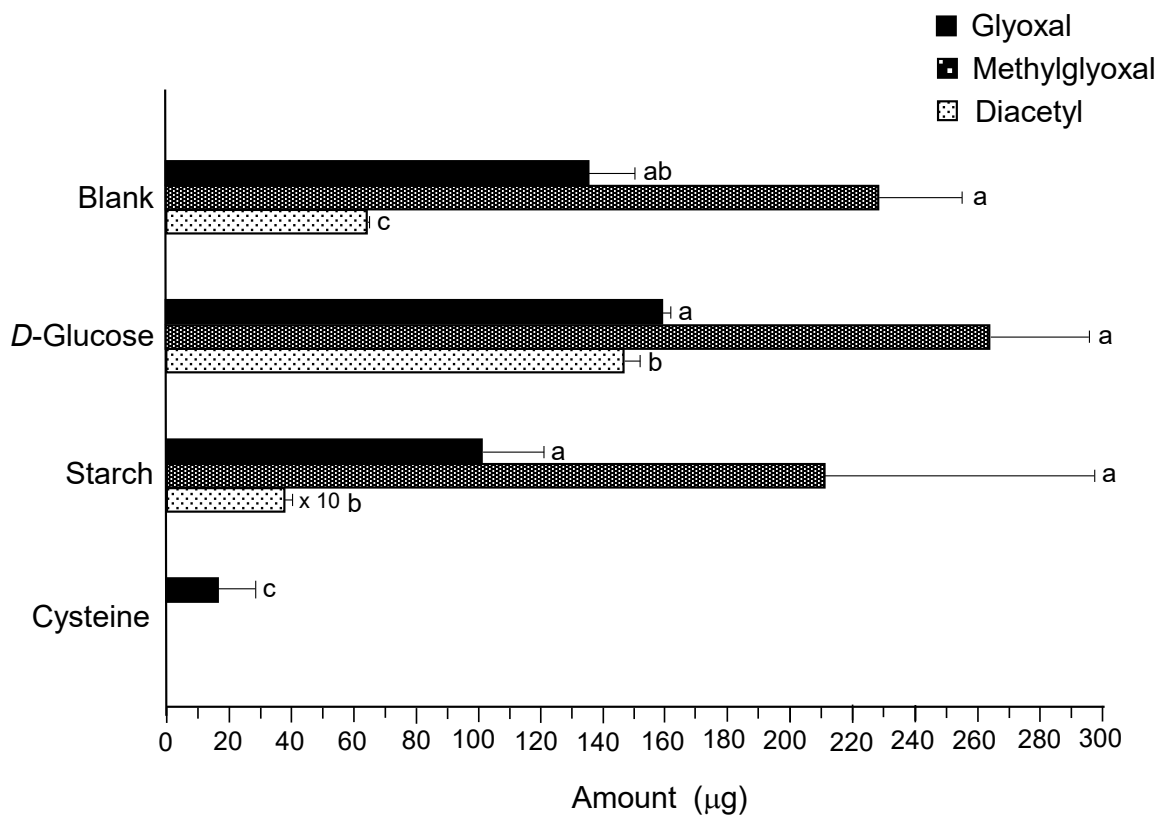

Figure 5: The results of the food matrix study. The values are mean ± standard deviation (n = 3).

The addition of BHT and vitamin E at 150°C brought the diacetyl amount from 30.35 ± 2.58 μg down to non-detectable. Also, the addition of Vitamin E reduced the amount of methylglyoxal formed from 17.41 ± 2.25 μg to a not detectable level. However, there is no significant difference among any of the three samples in the amount of glyoxal formation.

At 200°C, addition of BHT reduced the formation of glyoxal from 95.92 ± 6.19 μg to 72.82 ± 14.65 μg, methyl glyoxal to from 74.65 ± 11.44 μg to 59.27 ± 33.23 μg, and diacetyl from 33.99 ± 1.65 μg to not detectable. On the other hand, addition of vitamin E increased the formation of all three α-dicarbonyl compounds considerably. In particular, formation of diacetyl went from 33.99 ± 1.65 μg to 64.56 ± 57.03 μg.

At 250°C, moderate reductions of methylglyoxal from 227.74 ± 26.44 μg to 149.75 ± 18.29 μg and diacetyl from 63.85 ± 0.58 μg to 11.22 ± 19.44 μg were observed by the addition of BHT. On the other hand, glyoxal formation increased from 135.2 ± 15.14 μg to 160.94 ± 23.77 μg. Significant increases of glyoxal from 135.2 ± 15.14 μg to 421.77 ± 64.29 μg and diacetyl from 63.85 ± 0.58 μg to 229.04 ± 50.33 μg was exhibited by the addition of vitamin E. Also, a slight increase was seen in the case of methylglyoxal from 227.74 ± 26.44 μg to 232.99 ± 38.78 μg.

No clear trends were observed in the results of the antioxidant study. One major issue is that when an oil sample is treated with some vigorous conditions, such as high temperatures and prolonged heating periods, intensive oxidation occurs. Under such conditions, antioxidants do not work. In the present study, BHT seems to work at a relatively lower temperature at 150°C. Vitamin E reduced the formation of α-dicarbonyl compounds at 150°C. However, vitamin E increased the formation of α-dicarbonyl compounds slightly at 200°C and significantly at 250°C, suggesting that vitamin E itself degrades into α-dicarbonyl compounds upon oxidation. Therefore, use of antioxidants to prevent oxidation of cooking oils at high temperatures is not a productive avenue to pursue.

Food matrix study

Cooking oils are usually used together with foods, such as meats, vegetables, and rice. In particular, the use of cooking oils is mainly for deep fat frying. However, an actual food matrix is extremely complex. Therefore, some food components were used to prepare a simple food matrix to investigate the food matrix effect on the formation of α-dicarbonyl compounds in the present study.

Figure 5 shows the results of the food matrix study. The addition of *D*-glucose increased all three α-dicarbonyl compounds. Glyoxal increased from 135.2 ± 15.14 μg to 158.36 ± 2.36 μg. Methylglyoxal increased from 227.74 ± 26.44 μg to 263.12 ± 31.66 μg. Diacetyl increased from 63.85 ± 0.58 μg to 145.87 ± 4.96 μg. These results are reasonable because the sugar degradation by heat treatment produces many low molecular weight carbonyl compounds, including the three α-dicarbonyl compounds [12].

In the case of starch, slight reductions of glyoxal (from 135.2 ± 15.14 μg to 100.35 ± 19.54 μg) and methylglyoxal (from 227.74 ± 26.44 μg to 209.78 ± 87.04 μg) were observed. This may be because these two highly reactive μ-dicarbonyl compounds were absorbed or formed adducts with starch. On the other hand, the addition of starch increased diacetyl formation significantly from 63.85 ± 0.58 μg to 376.02 ± 26.3 μg. This could possibly be some starch degrading into diacetyl.

Very interesting results were obtained from the addition of cysteine. Considerable reductions of glyoxal (from 135.2 ± 15.14 μg to 16.4 ± 11.94 μg), methylglyoxal (from 227.74 ± 26.44 μg to not detected), and diacetyl (from 63.85 ± 0.58 μg to not detected) were observed. It is well known that cysteamine, which is a decarboxylated compound of cysteine, forms stable derivatives with reactive aldehydes and ketones. It has been used for analysis of reactive aldehydes, such as formaldehyde and acetaldehyde [1]. Therefore, cysteine likely trapped these α-dicarbonyl compounds directly, or formed cysteamine and then trapped them.

It is not easy to rationalize the results of the present matrix study. Additional investigations into the role of the food matrix in the formation of low molecular weight carbonyl compounds is in order.

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