Comparison of α-dicarbonyl compound contents in alcoholic beverages.

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Abstract

**Purpose:** In the process of producing advanced glycation end products (AGEs), α-dicarbonyl compounds (α-DCs) act as intermediates for AGEs. In this study, we measured the amount of 3-deoxyglucosone (3-DG), glyoxal (GO), and methylglyoxal (MGO) as α-DCs contained in alcoholic beverages.

**Methods:** As samples, 30 commercially available alcoholic beverages (18 brewages: Japanese sake, beer, red wine, white wine, and sherry, 15 distilled spirits: whiskey, shochu, gin, vodka, and rum) were used. α-DCs in each sample were labeled with 2,3-diaminonaphthalene (DAN) and measured by reversed phase high performance liquid chromatography (HPLC).

**Results:** Concentrations of 3-DG and GO in alcoholic beverages were higher in brewages than in distilled spirits. The concentration of 3-DG was the highest in brewages in the order of beer (1,048.7 ± 120.6 μg/mL), white wine (216.7 ± 98.5 μg/mL), red wine (177.0 ± 59.8 μg/mL), sherry (107.3 ± 7.6 μg/mL), and sake (51.0 ± 4.8 μg/mL). The 3-DG concentration of distilled spirits was 4.8 ± 13.8 μg/mL. The GO concentration of brewages was in the order of sherry (6.5 ± 3.2 μg/mL), beer (4.5 ± 0.8 μg/mL), red wine (3.3 ± 0.7 μg/mL), white wine (2.7 ± 1.9 μg/mL), and sake (0.9 ± 0.3 μg/mL). The GO concentration of the distilled spirits was 1.2 ± 1.8 μg/mL. Among brewages, α-DC concentration of sake was low.

**Conclusions:** Alcoholic beverages contained 3-DG, GO, and MGO as α-DCs, the contents of which was low in distilled spirits. In brewages, the content of 3-DG and GO in sake was low. Alcoholic beverages with less glycative stress could be sake in distilled spirits or brewages, however, proper intake should be strictly observed.

**KEY WORDS:** advanced glycation end products (AGEs), 3-deoxyglucosone, glyoxal, methylglyoxal, Japanese sake

Introduction

The glycative reaction in the living body is caused by an irreversible reaction between reducing sugar and protein. α-Dicarbonyl compounds (α-DCs) are generated by dehydration/hydrolysis of Amadori compounds and cleavage between carbons, act as intermediates in the glycative stress-induced reactions. α-DCs in vivo include glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosone (3-DG), which have two carbonyl bonds (C = O) as shown in Fig. 1. α-DCs react with amino acids when heated or exposed to ultraviolet light, causing Strecker’s decomposition and producing carbon dioxide, aldehydes, pyrazines, and carbonylated proteins.

These compounds change to intermediates, structurally have an aldehyde base (-CHO), and thus recognized as α-DCs, α-dicarbonyl compounds; MW, molecular weight.

Fig. 1. Structures of α-DCs; 3-DG, GO and MGO.

a) 3-deoxyglucosone (3-DG); MW, 162.14 g/mol; density, 1.406 g/mL.

b) Glyoxal (GO); MW, 58.04 g/mol; density, 1.270 g/mL.

c) Methylglyoxal (MGO); MW, 72.06 g/mol; density, 1.046 g/mL. α-DCs, α-dicarbonyl compounds; MW, molecular weight.
aldehyde in vivo, resulting in being involved in the production of advanced glycation end products (AGEs)\(^3\). Accumulation of AGEs in living tissues is a factor that causes functional deterioration of proteins and, therefore, has been reported to be involved in the onset of diabetes, osteoporosis, or other degenerative disease\(^4\).

Food has three functions related to energy source, palatability, and biological regulation. Foods with strong functions related to biological regulation are called functional foods. In recent years, various functional foods have been developed with various materials that act to suppress the glycative reaction\(^5,6\). Among these are fermented foods, which are processed by fermenting food materials by the action of microorganisms or chemical reactions. Japanese sake, one of the fermented foods, is a food product obtained by milling raw rice and then fermenting it by adding koji mold and yeast. Sake has already been reported to have functional components of sake and its mechanism of action\(^5,6\), while alcoholic beverages cause various organ disorders when their intake increases\(^6\). As affairs, there are few reports on α-DCs contained in alcoholic beverages\(^11,12\). In this study, the amount of α-DCs contained in alcoholic beverages was measured and the content was compared according to the type of beverage.

### Materials and methods

#### Materials

Alcoholic drinks used as samples, 33 items, were purchased at liquor stores in Kyoto, Japan. The purchased alcoholic beverages were classified into 18 brewages (six sake, three beer, three red wine, three white wine, and three sherry) and 15 distilled spirits (three whiskey, three shochu, three gin, three vodka, and three rum) as shown in Table 1.

#### Measurement of solid content

The solid content concentration (mg/mL) of the sample was calculated by adding 5 mL of the sample to an aluminum tray and measuring the weight before and after drying at 120°C for 2 hours followed by calculating from the change value.

### Table 1. The sample list of alcoholic beverages

<table>
<thead>
<tr>
<th>ID</th>
<th>Liquor type</th>
<th>Classification</th>
<th>Sample name</th>
<th>Product name</th>
<th>Manufacturer</th>
<th>Alcohol percentage</th>
<th>Solid content (mg/mL)</th>
<th>Sugar content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S5</td>
<td>Seishu</td>
<td>A</td>
<td>Konteki</td>
<td>Higashiya Shuzo (Kyoto, Japan)</td>
<td>16%</td>
<td>46.2</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>Seishu</td>
<td>B</td>
<td>Hana no Izanai (Ginjo type)</td>
<td>Shoutoku Shuzo (Kyoto, Japan)</td>
<td>12%</td>
<td>28.0</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>S7s</td>
<td>Japanese sake (Seishu)</td>
<td>C</td>
<td>Hana no Izanai (Summer limited edition) (Ginjo type)</td>
<td>Shoutoku Shuzo (Kyoto, Japan)</td>
<td>12%</td>
<td>33.0</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>S8</td>
<td>Seishu</td>
<td>D</td>
<td>Densho Yamaha Shikomi Tamanohikari (Ginjo type)</td>
<td>Tamanohikari Shuzo (Kyoto, Japan)</td>
<td>16%</td>
<td>45.5</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>S11</td>
<td>Seishu</td>
<td>E</td>
<td>Tomiou Tanshu Yamadanishiki (Ginjo type)</td>
<td>Kitagawa Honke (Kyoto, Japan)</td>
<td>15%</td>
<td>47.6</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>S15</td>
<td>Brewages</td>
<td>F</td>
<td>Eikun Koto Sen-nen (Ginjo type)</td>
<td>Saito Shuzo (Kyoto, Japan)</td>
<td>15%</td>
<td>52.3</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>Beer</td>
<td>A</td>
<td>Ichiban Shibori</td>
<td>Kirin Brewery (Tokyo, Japan)</td>
<td>5%</td>
<td>167.1</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>Beer</td>
<td>B</td>
<td>Super Dry</td>
<td>Asahi Breweries (Tokyo, Japan)</td>
<td>5%</td>
<td>161.5</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>Beer</td>
<td>C</td>
<td>Black Label</td>
<td>Sapporo Breweries (Tokyo, Japan)</td>
<td>5%</td>
<td>168.9</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>WR1</td>
<td>Red wine</td>
<td>A</td>
<td>World Selection</td>
<td>Mercian (Tokyo, Japan)</td>
<td>12%</td>
<td>121.5</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>WR2</td>
<td>Red wine</td>
<td>B</td>
<td>Vina Maipo Cabernet Sauvignon</td>
<td>Vina Maipo (Santiago, Chile)</td>
<td>12%</td>
<td>136.1</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>WR3</td>
<td>Red wine</td>
<td>C</td>
<td>Aires Andinos</td>
<td>Bodegas La Rosa (Mendoza, Argentina)</td>
<td>12%</td>
<td>128.0</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>
**α-Dicarbonyl Compounds in Alcoholic Beverages**

| WW1 | White wine | White wine A | Tree Bear | Lion (Sydney, Australia) | 13% | 143.9 | 7.7 |
| WW2 | White wine | White wine B | Miramonte | Miramonte Sauvignon Blanc-Semillón (Valle Central, Chile) | 12% | 92.0 | 6.9 |
| WW3 | White wine | White wine C | Grande Polaire Okayama Muscatbailey | Sapporo Breweries (Tokyo, Japan) | 11% | 241.8 | 9.1 |
| WS1 | Sherry A | Sherry A | González Tío Pepe | González Byass (Jerez de la Frontera, Spain) | 15% | 54.6 | 7.2 |
| WS2 | Sherry B | Sherry B | Manzanilla La Sanluquena | Bodegas Teresa Rivero (Sanlúcar de Barrameda, Spain) | 15% | 68.3 | 7.6 |
| WS3 | Sherry C | Sherry C | Sandeman Fino | Sandeman (Jerez de la Frontera, Spain) | 15% | 66.7 | 7.4 |
| W1 | Whisky A | Whisky A | Suntory Yamazaki | Suntory (Osaka, Japan) | 40% | 3.5 | 15.1 |
| W2 | Whisky B | Whisky B | Chita | Suntory (Osaka, Japan) | 43% | 2.9 | 15.8 |
| W3 | Whisky C | Whisky C | Jack Daniel's | Jack Daniel's (Lynchburg, USA) | 40% | 5.2 | 15.1 |
| J1 | Potato shochu | Potato shochu | Kuro Kirishima | Kirishima Shuzo (Miyazaki, Japan) | 25% | 0.2 | 10.0 |
| J2 | Barley shochu | Barley shochu | Iichiko | Sanwa Shurui (Oita, Japan) | 20% | 0.2 | 7.8 |
| J3 | Rice shochu | Rice shochu | Chonma | Hakutaka (Nada, Hyogo, Japan) | 25% | 0.1 | 9.9 |
| G1 | Gin A | Gin A | Beefeater | Beefeater (London, UK) | 47% | 0.0 | 16.4 |
| G2 | Gin B | Gin B | Dry Gin | Suntory (Osaka, Japan) | 40% | 0.0 | 14.8 |
| G3 | Gin C | Gin C | Wilkinson | Nikka Whisky (Yoichi, Hokkaido, Japan) | 37% | 0.1 | 14.0 |
| V1 | Vodka A | Vodka A | Skyy | Skyy Spirits (San Francisco, USA) | 40% | 0.0 | 14.7 |
| V2 | Vodka B | Vodka B | Suntory Vodka | Suntory (Osaka, Japan) | 40% | 0.0 | 14.6 |
| V3 | Vodka C | Vodka C | Wilkinson | Nikka Whisky (Yoichi, Hokkaido) | 40% | 0.4 | 14.7 |
| R1 | Rum A | Rum A | Myers's Rum | Sazerac (Metairie, USA) | 40% | 13.9 | 14.9 |
| R2 | Rum B | Rum B | Dover White | Dover (Tokyo, Japan) | 45% | 0.5 | 15.7 |
| R3 | Rum C | Rum C | Dover 45 | Dover (Tokyo, Japan) | 45% | 7.4 | 15.8 |

**Measurement of sugar content**

The sugar content of the sample was measured using a pocket sugar content meter (PL-1: Atago, Tokyo, Japan).

**α-DC measurement in alcoholic beverages**

For α-DCs in alcoholic beverages, 3-DG, GO, and MGO concentrations were measured according to previous reports (3). For sample pretreatment, add 100 μL of each sample, 155 μL of purified water, and 75 μL of 200 mmol/L phosphate buffer, then add 170 μL of 6% perchloric acid followed by centrifuge at 15,000 rpm for 10 minutes. For labeling α-DCs by 2,3-diaminonaphthalene (DAN), 400 μL of the supernatant after centrifugation was mixed with 350 μL of saturated sodium hydrogen carbonate solution, and then 50 μL of 50% methanol containing 1 mg/mL DAN was added and reacted at 4°C for 22 hours. The labeled solution was centrifuged at 4°C, 15,000 rpm for 10 minutes, and the supernatant was used as a sample solution for HPLC analysis. Table 2 shows the analytical conditions for 3-DGL, GO, and MGO by HPLC.
The measurement was performed 3 times for each sample (n = 3), and the results are expressed as the average value ± standard deviation. Statistical analysis was performed by t-test for comparison between two groups and Tukey-Kramer multiple comparison test for comparison between three or more groups. The significance level was determined by a two-sided test to be significant with a level of less than 5%.

Results

1. Solid content and sugar content

Table 1 shows the solid content and sugar content of the alcoholic beverages used as samples. The solid content of brewages (100.2 ± 60.6 µg/mL, n = 18) was 43.7 times higher than that of distilled spirits (2.3 ± 3.9 mg/mL, n = 15, p < 0.001). The solid content of brewages in the order from the highest is beer (165.8 ± 3.9 mg/mL, n = 3), white wine (159.2 ± 76.1 mg/mL, n = 3), red wine (128.5 ± 7.3 mg/mL, n = 3), sherry (63.2 ± 7.5 mg/mL, n = 3), and sake (42.9 ± 9.4 mg/mL, n = 6). The solid content of distilled spirits in the order is whiskey (15.3 ± 0.4%, n = 3), rum (7.3 ± 0.7%, n = 3), shochu (9.2 ± 1.2%, n = 3), and vodka (14.0 ± 2.6%, n = 15) than in brewages (8.1 ± 1.7%, n = 18, p < 0.001). The sugar content of brewages in the order is sake (9.9 ± 1.6%, n = 6), white wine (7.9 ± 1.1%, n = 3), red wine (7.4 ± 0.3%, n = 3), sherry (7.4 ± 0.2%, n = 3), and beer (6.3 ± 0.1%, n = 3). The sugar content of distilled spirits in the order is whiskey (15.3 ± 0.4%, n = 3), rum (15.5 ± 0.5%, n = 3), gin (15.1 ± 1.2%, n = 3), vodka (14.7 ± 0.1%, n = 3), and shochu (9.2 ± 1.2%, n = 3).

2. 3-DG concentration in alcoholic beverages

3-DG concentrations differed among the samples, nevertheless the average value of brewages (275.3 ± 366.7 µg/mL, n = 18) was 57.8% higher than that of distilled spirits (4.8 ± 13.8 µg/mL, n = 15, p < 0.001, Fig. 2). The concentration of 3-DG in brewages in the order was beer (1,048.7 ± 120.6 µg/mL, n = 3), white wine (216.7 ± 98.5 µg/mL, n = 3), red wine (177.0 ± 59.8 µg/mL, n = 3), sherry (159.2 ± 76.1 µg/mL, n = 3), and sake (107.3 ± 7.6 µg/mL, n = 3). The 3-DG concentration of distilled spirits was high in rum (19.4 ± 30.1 µg/mL, n = 3), whiskey (4.2 ± 2.1 µg/mL, n = 3), and shochu (0.2 ± 0.3 µg/mL, n = 3), while it was not noted in gin or vodka.

3. GO concentration in alcoholic beverages

The GO concentration was lower than that of 3-DG, and the average value of brewages (3.1 ± 2.4 µg/mL, n = 18) was higher than that of distilled liquor (1.2 ± 1.8 µg/mL, n = 15), being 2.7 times higher (p < 0.05, Fig. 3). The GO concentration of brewages in the order was sherry (6.5 ± 3.2 µg/mL, n = 3), beer (4.5 ± 0.8 µg/mL, n = 3), red wine (3.3 ± 0.7 µg/mL, n = 3), white wine (2.7 ± 1.9 µg/mL, n = 3) and sake (0.9 ± 0.3 µg/mL, n = 6). 3-DG of distilled spirits were recognized in whiskey (3.4 ± 1.3 µg/mL, n = 3) and rum (2.4 ± 2.5 µg/mL, n = 3), slight in shochu, but not noted in gin or vodka.

4. MGO concentration in alcoholic beverages

The MGO concentration was lower than that of 3-DG, similar to the concentration of GO, and there was no significant difference in the mean values between brewages (3.7 ± 2.9 µg/mL, n = 18) and distilled spirits (2.0 ± 3.5 µg/mL, n = 15, Fig. 4). MGO concentration of brewages in the order is white wine (6.7 ± 3.8 µg/mL, n = 3), red wine (5.8 ± 0.3 µg/mL, n = 3), sherry (4.9 ± 2.6 µg/mL, n = 3), and sake (1.0 ± 0.4 µg/mL, n = 6). MGO concentrations in distilled spirits were recognized in whiskey (8.2 ± 2.9 µg/mL, n = 3) and rum (1.3 ± 1.5 µg/mL, n = 3), however, MGO was found only slightly in shochu (0.2 ± 0.0 µg/mL, n = 3), gin (0.1 ± 0.1 µg/mL, n = 3), and vodka (0.1 ± 0.1 µg/mL, n = 3).

5. α-DC content by type of alcoholic beverage

The alcoholic beverages used as samples were classified into 10 categories, and the concentrations of 3-DG, GO, and MGO were compared for each category. The 3-DG concentration of beer was higher than that of the other 9 categories (p < 0.001, Fig. 5). White wine contained higher 3-DG than sake, whiskey, shochu, gin, vodka, and rum (p < 0.001), while red wine has higher 3-DG than whiskey, shochu, gin, vodka, and rum (p < 0.05). The 3-DG value of sake was lower than that of white wine (p < 0.001).

The GO concentration of sherry was higher than that of sake, shochu, gin, vodka (p < 0.001), and rum (p < 0.05, Fig. 6). Beer contained higher GO than sake (p < 0.001), shochu, gin, and vodka (p < 0.05). The GO value of sake was

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Table 2. HPLC condition.

| Column | Unison UK-Phenyl, 75 × 3 mm I.D. (Intakt, Kyoto, Japan) |
| Eluent | A: 2.5 mmol/L phosphate, B: acetonitrile |
| Gradient | 0-30 min (0-10% B), 30-42 min (10% B), 42-47 min (10-70% B) |
| Detector | Excitation: 271 nm, Emission: 503 nm |
| Flow rate | 1.0 mL/min |
| Column temperature | 40 °C |
| Injection volume | 20 µL |
**Fig. 2.** 3-DG content in alcoholic beverage.

Results are expressed as mean ± standard deviation in the triplet measurement. 3-DG, 3-deoxyglucosone.

**Fig. 3.** GO content in alcoholic beverage.

Results are expressed as mean ± standard deviation in the triplet measurement. GO, glyoxal.
Fig. 4. MGO content in alcoholic beverage.
Results are expressed as mean ± standard deviation in the triplet measurement. MGO, methylglyoxal.

Fig. 5. Comparison of 3-DG content by a type of alcoholic beverage.
Results are expressed as mean ± standard deviation. ** p < 0.01 by Tukey-Kramer test, n = 3 each except sake (n = 6). 3-DG, 3-deoxyglucosone.
Fig. 6. Comparison of GO content by a type of alcoholic beverage.
Results are expressed as mean ± standard deviation, ** p < 0.01 by Tukey-Kramer test, n = 3 each except sake (n = 6). GO, glyoxal.

Fig. 7. Comparison of MGP content by a type of alcoholic beverage.
Results are expressed as mean ± standard deviation, *, ** p < 0.01 by Tukey-Kramer test, n = 3 each except sake (n = 6). MGO, methylglyoxal.
Discussion

1. Manufacturing method of alcoholic beverage, solid content, and sugar content

Generally, brewages are liquor obtained by alcoholic fermentation of raw materials such as yeast. Distilled spirits are liquor obtained by distilling brewed liquor (brewages). In this study, the solid content was 43.7 times higher in brewages than in distilled spirits (p < 0.001), while the sugar content of brewages was 1.7 times lower than that of distilled spirits (p < 0.001). The solid content of brewages is considered to be derived from sugars produced during the brewing process in which the material is alcohol-fermented with yeast, and the components that are produced from the grain of the material during the fermentation process. Typically, in distilled spirits, as the distillation of the original raw liquor is repeated, the alcohol content increases and the solid content is reduced due to removal of the sugars from the raw materials. In this measurement, the sugar content of distilled spirits was higher than that of brewages. This may be due to the influence of the alcohol concentration on the sugar content measurement with a sugar content meter. As a principle, the sugar content meter calculates the sugar concentration from the refractive index of the sample solution. The alcohol content of distilled spirits was 37.8 ± 0.08% (n = 15), which was 3.1 times higher than that of brewages at 12.1 ± 0.04% (n = 18). As a matter of course, a positive correlation was noted between the alcohol content and the sugar content of all samples (y = 24.245x + 4.999, r = 0.958, n = 33).

2. Aldehyde content of sake

There are some reports on the acetaldehyde content of sake. The acetaldehyde contained in sake is produced by the oxidation of ethanol produced during the brewing process. That is, alcohol is sequentially changed from ethanol to acetaldehyde, and finally to acetic acid by the oxidation of ethanol produced during the brewing process. That is, alcohol is sequentially changed from ethanol to acetaldehyde, and finally to acetic acid by the oxidation of ethanol produced during the aging period. Typically, in distilled spirits, due to removal of the sugars from the raw materials. In this study, the 3-DG amount of sake samples (n = 6) was 14.2 ± 0.03 μmol/L, where an 8.2 times difference was observed. In the whisky distillery, the manufacturers use copper stills (distillation apparatus), called pot stills. It is known that glycate reaction occurs by heating in the distillation process, and β-damascenone and fural are produced in the oxidation product derived from glycated materials during aging. In this study, we found that alcoholic beverages contained 3-DG, GO, and MGO as α-DCs.

MGO concentration in sake

The MGO content in alcoholic beverages was lower than that of 3-DG, and was recognized in white wine (5.8 ± 2.9 μg/mL, n = 3), white wine (5.8 ± 3.8 μg/mL, n = 3), and red wine (5.8 ± 0.3 μg/mL, n = 3). In the whisky distillery, the manufacturers use copper stills (distillation apparatus), called pot stills. It is known that glycate reaction occurs by heating in the distillation process, and β-damascenone and fural are produced. MGO in whiskey may be an antioxidation product derived from glycated materials during the distillation process. One of the characteristics of sake is that the amount of GO is small.

3. Regarding the safety of α-DCs contained in food and beverage

In this study, we found that alcoholic beverages contained 3-DG, GO, and MGO as α-DCs. Manuka honey and coffee are known to contain MGO. Manuka honey is a honey collected by bees from the nectar of Manuka (Leptospermum scoparium), native to New Zealand, and has a unique flavor like a crude drug. Manuka means "resurrection tree" or "healing tree" in the words of native
Maori people. Manuka honey has already been reported to have antibacterial action\(^{28}\), antiviral action\(^{19}\), wound healing promotion\(^{20}\), and plaque prevention action\(^{21}\). Manuka honey contains 80 mg/kg (80 μg/g) to 1,200 mg/kg (1,200 μg/g) of MGO\(^{22, 23}\). It is said that the higher the MGO content, the better the quality of Manuka honey. On the other hand, coffee contains 50 to 70 μmol/L of MGO. MGO of coffee differs depending on the type of beans and the extraction method. Espresso contains 230.9 μmol/L (16.6 μg/mL) of MGO\(^{24}\). The concentration of MGO in alcoholic beverages is 15 μg/mL or less, which is lower than that of manuka honey or espresso.

Aldehydes and α-DCs such as GO\(^{25}\), which are associated with glycative stress, may cause DNA damage and induce genetic mutations and teratogenicity if the intake amount is high\(^{26, 27}\). The generation of 3-DG \textit{in vivo} increases the risk of teratogenicity\(^{28}\). Excessive intake of alcoholic beverages causes DNA damage in squamous cells and increases the onset risk of esophageal cancer\(^{29}\), which becomes higher particularly by the intake of the liquor with high alcohol content.

There are various indicators for food safety evaluation. The no observed adverse effect level (NOAEL) is the dose level at which no adverse effects were observed in animal toxicity studies. Generally, a 1/100 concentration (dose) of NOAEL is approved as an acceptable daily intake (ADI) in humans. ADI is the amount by which a person is considered to have no adverse health effects if they continue to eat every day for a lifetime. It is customary to take one-third of ADI for safe daily intake.

GO is contained, as well as in alcoholic beverages, in fermented foods such as miso and yogurt (0.63 to 4.2 mg/kg), bakery foods such as bread (0.07 to 1.6 mg/kg), vegetable food materials (3 to 14 mg/kg), and cooking oil (up to 6.5 mg/kg).

Estimating the medium-term toxicity of GO, NOAEL is 100 to 130 mg/kg bw/day in Europe\(^{30}\); ADI 1.0 to 1.3 mg/kg bw/day, one-third of that amount is 0.3 to 0.4 mg/kg bw/day, thus indicating the acceptable dose may be 15 to 20 mg/day in a person weighing 50 kg. However, safety evaluation regarding carcinogenicity has not been performed yet.

Assuming the reproductive and developmental toxicity of GO, the NOEAL of maternal rats is 25 mg/kg/day\(^{30}\). Therefore, the NOEAL of GO is about 3 to 4 mg/day when the safety is strictly estimated. The intake of 300 mL of sake, 120 mL of beer, 50 mL of wine, and 40 mL of whiskey is calculated to reach the NOEAL of GO. For this reason, pregnant women should refrain strictly from drinking alcohol. The safety standard for GO intake in the EU is 0.17 mg/kg bw/day. Provided that, this calculation is based on a daily intake of alcohol for one week. Consequently, if an individual observes the intake after setting a liver-free day, the allowable amount will be a little bit larger than this.

### Conclusion

Alcoholic beverages contained α-DCs, 3-DG, GO, and MGO. Among them, distilled spirits were shown to have a low α-DCs content, while, in brewages, the content of 3-DG and GO in sake was low. The alcoholic beverage with a low glycative stress could be distilled spirits or sake in brewages. However, proper intake should be strictly observed.

### Acknowledgment

The part of this study was presented at the 18th Meeting of Glycative Stress Research on August 31, 2019, Kyoto, Japan.

### Conflict of Interest Statement

The authors claim no conflict of interest in this study.

### Reference