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Original article

Comparison of α-dicarbonyl compound contents in alcoholic beverages.

Tatsushi Kimoto, Wakako Takabe, Masayuki Yagi, Mari Ogura, Yoshikazu Yonei

Anti-Aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

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 ± 44.8 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 endproducts (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 [1,2].

These compounds change to intermediates, structurally have an aldehyde base (-CHO), and thus recognized as

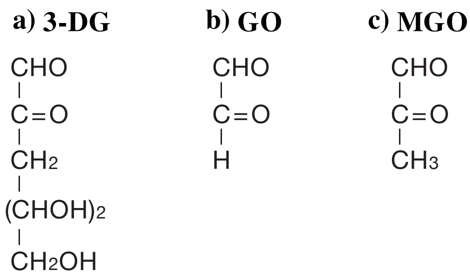

Fig. 1. **Structures of α-DCs; 3-DG, GO and MGO.**
a) 3-deoxyglucosone (3-DG); MW, 162.14 g/mol; density, 1.406 g/mL. **b)** Glyoxal (GO); MW, 58.04 g/mol; density, 1.270 g/mL. **c)** Methylglyoxal (MGO); MW. 72.06 g/mol; density, 1.046 g/mL. α-DCs, α-dicarbonyl compounds; MW, molecular weight.

Contact Address: Professor Masayuki Yagi, PhD
Anti-Aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences, Doshisha University
1-3 Tatara Miyakodani, Kyotanabe, Kyoto, 610-0394 Japan
TEL/FAX: +81-774-65-6394 e-mail: myagi@mail.doshisha.ac.jp
Co-authors: Kimoto T, ta.chan027@gmail.com;
Takabe W, wtakabe@mail.doshisha.ac.jp; Ogura M, m-ogura@po.kbu.ac.jp;
Yonei Y, yyonei@mail.doshisha.ac.jp

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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 functionalities such as prevention of hypertension, prevention of osteoporosis, suppression of cholesterol absorption, and antioxidant effect[7]. There is a saying in Japan that "liquor is a hundred medicine", conveying a message that there is an advantage if you drink a proper amount of alcohol. There have been many reports on the identification of functional components of sake and its mechanism of action[8,9], while alcoholic beverages cause various organ disorders when their intake increases[10]. 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

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

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

Table 2. HPLC condition.

Column:	Unison UK-Phenyl, 75 x 3 mm I.D. (Imtakt, 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, Enission: 503 nm
Flow rate:	1.0 mL/min
Column temperature:	40°C
Injection volume:	20 µL

Statistical analysis

The measurement was performed 3times 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 mg/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 rum (7.3 ± 6.7 mg/mL, n = 3), whiskey (3.9 ± 1.2 mg/mL, n = 3), shochu (0.2 ± 0.1 mg/mL, n = 3), gin (0.1 ± 0.2 mg/mL, n = 3), and vodka (0.03 ± 0.1 mg/mL).

The sugar content was 1.7 times higher in distilled spirits (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 (107.3 ± 7.6 µg/mL, n = 3), and sake (51.0 ± 44.8 µg/mL, n = 6). 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 was 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), followed by beer (2.6 ± 0.5 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

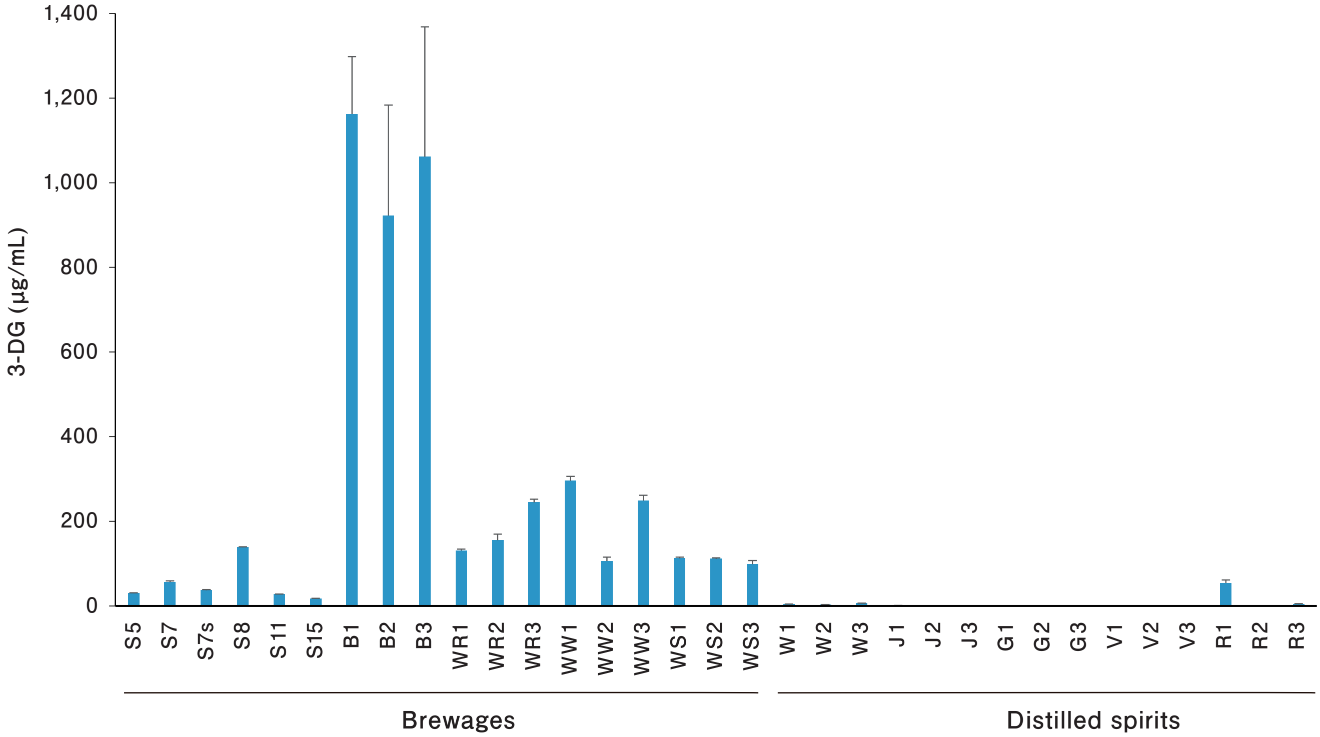

Fig. 2. **3-DG content in alcoholic beverage.**

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

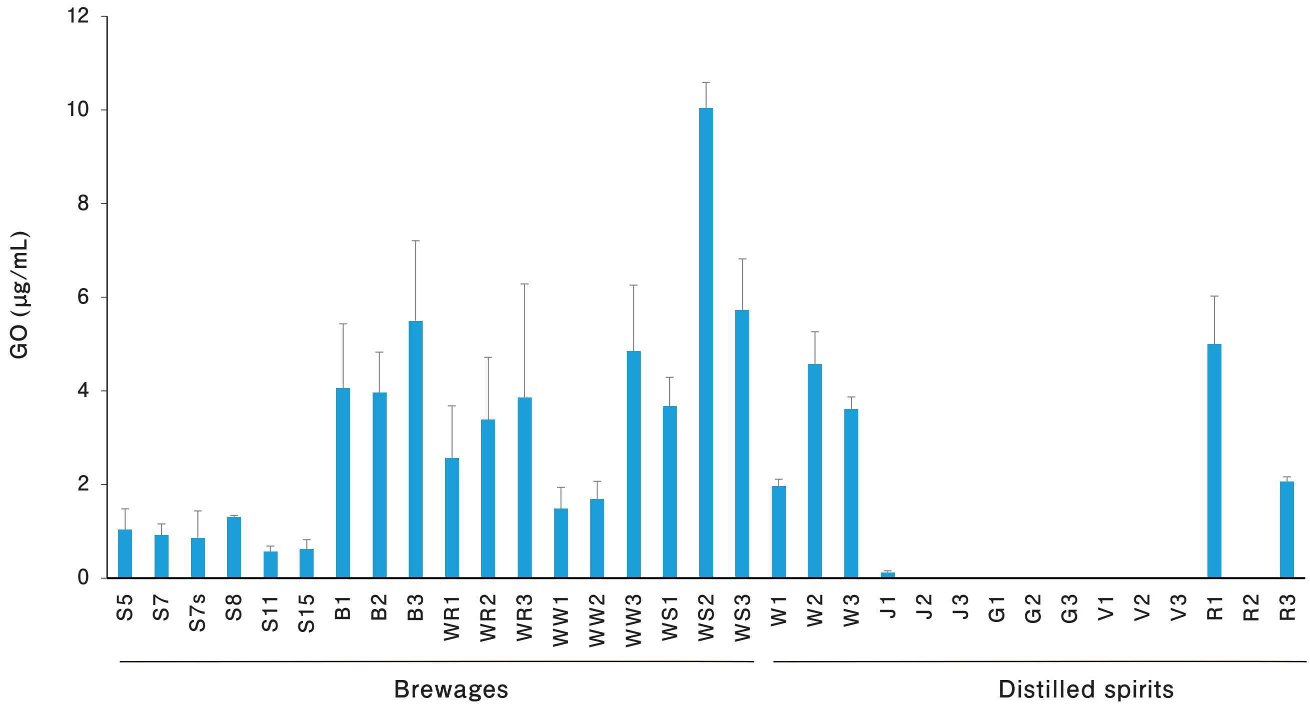

Fig. 3. **GO content in alcoholic beverage.**

Results are expressed as mean ± standard deviation in the triplet measurement. GO, glyoxal.

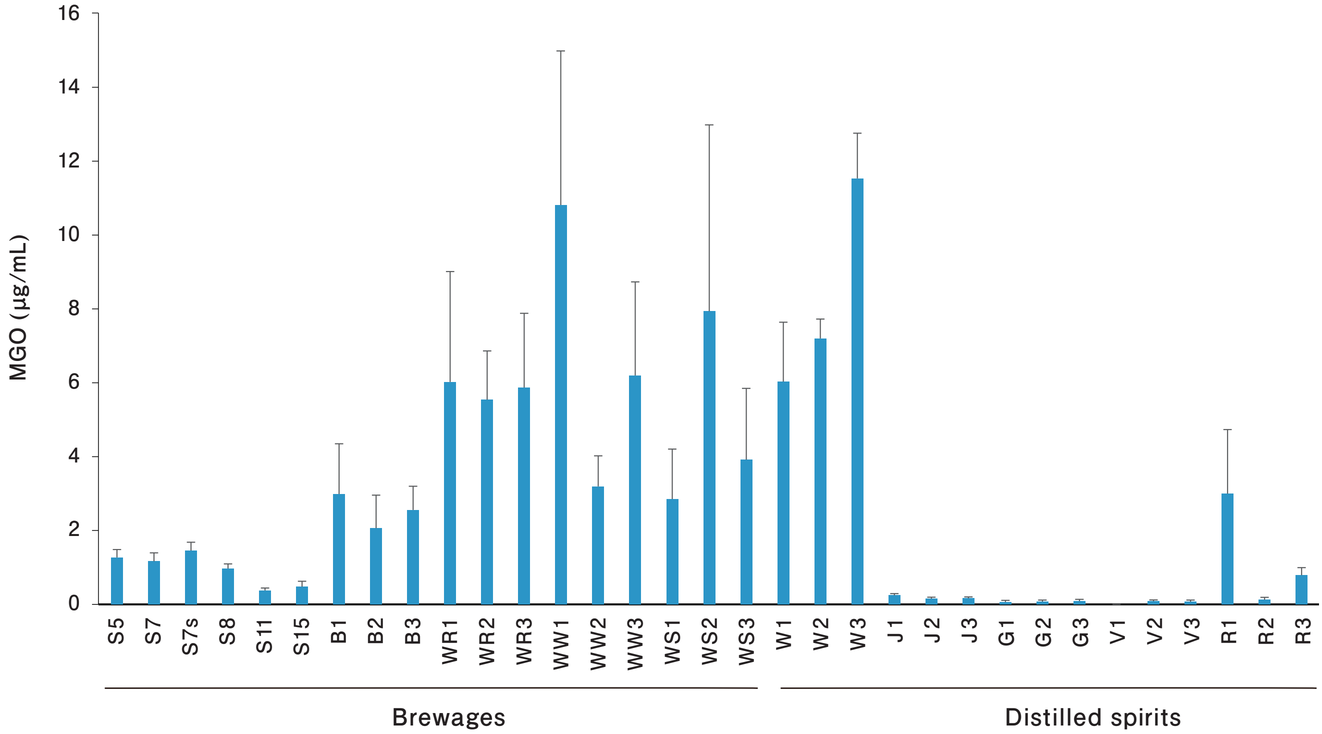

Fig. 4. **MGO content in alcoholic beverage.**

Results are expressed as mean ± standard deviation in the triplet measurement. MGO, methylglyoxal.

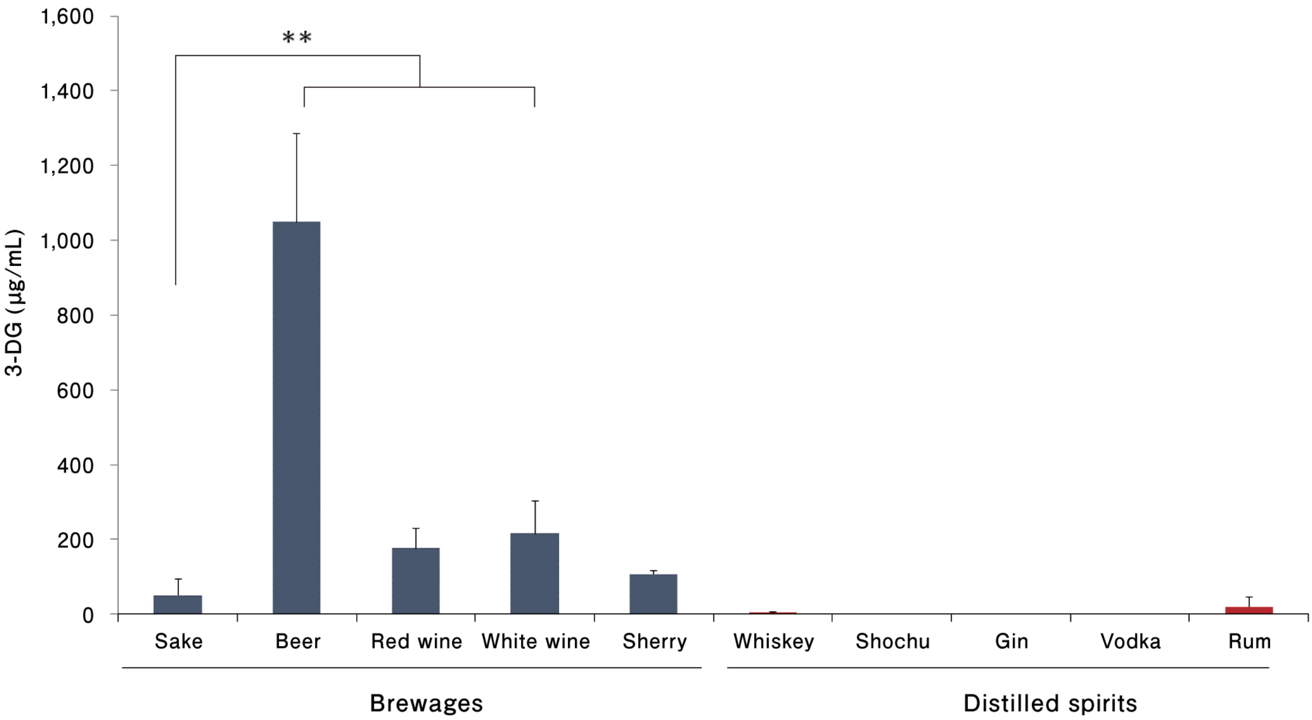

Fig. 5. **Comparison of 3-DG content by a type of alcoholic beverage.**

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.

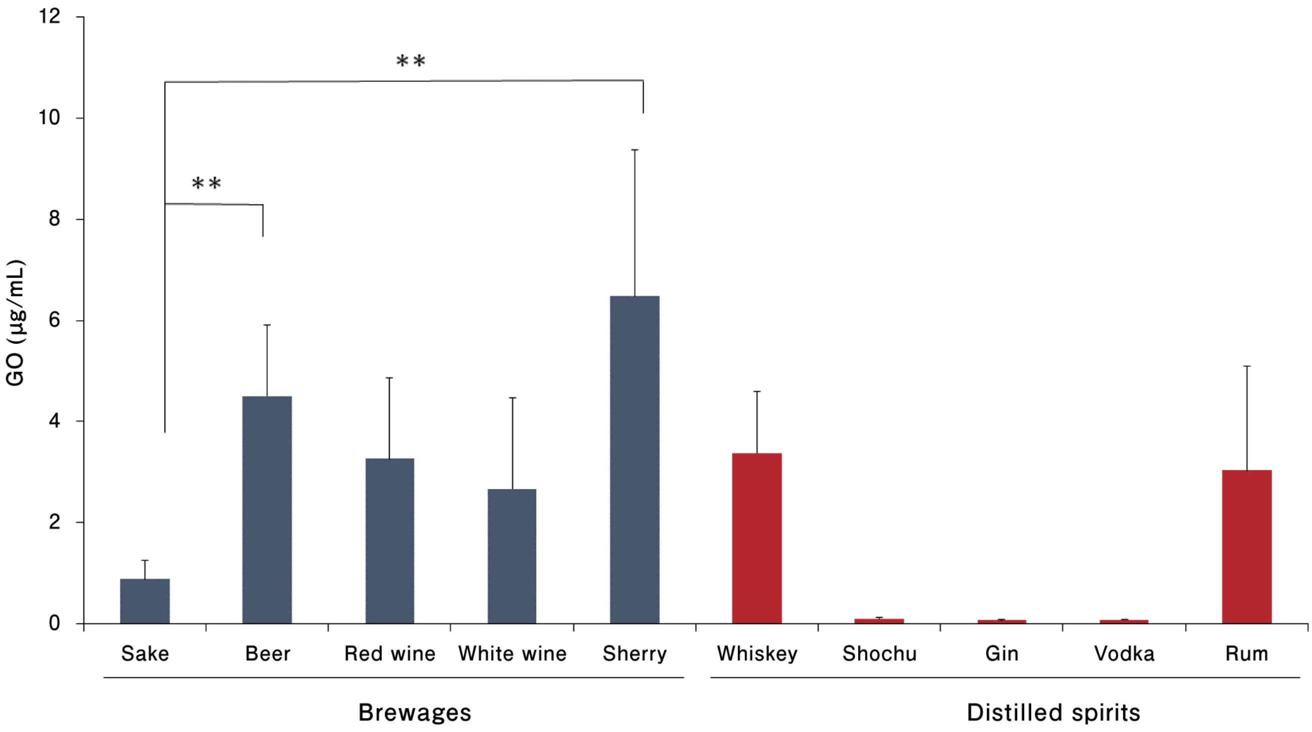

Fig. 6. **Comparison of GO content by a type of alcoholic beverage.**

Results are expressed as mean ± standard deviation, ** $p < 0.01$ by Tukey-Kramer test, n = 3 each except sake (n = 6). GO, glyoxal.

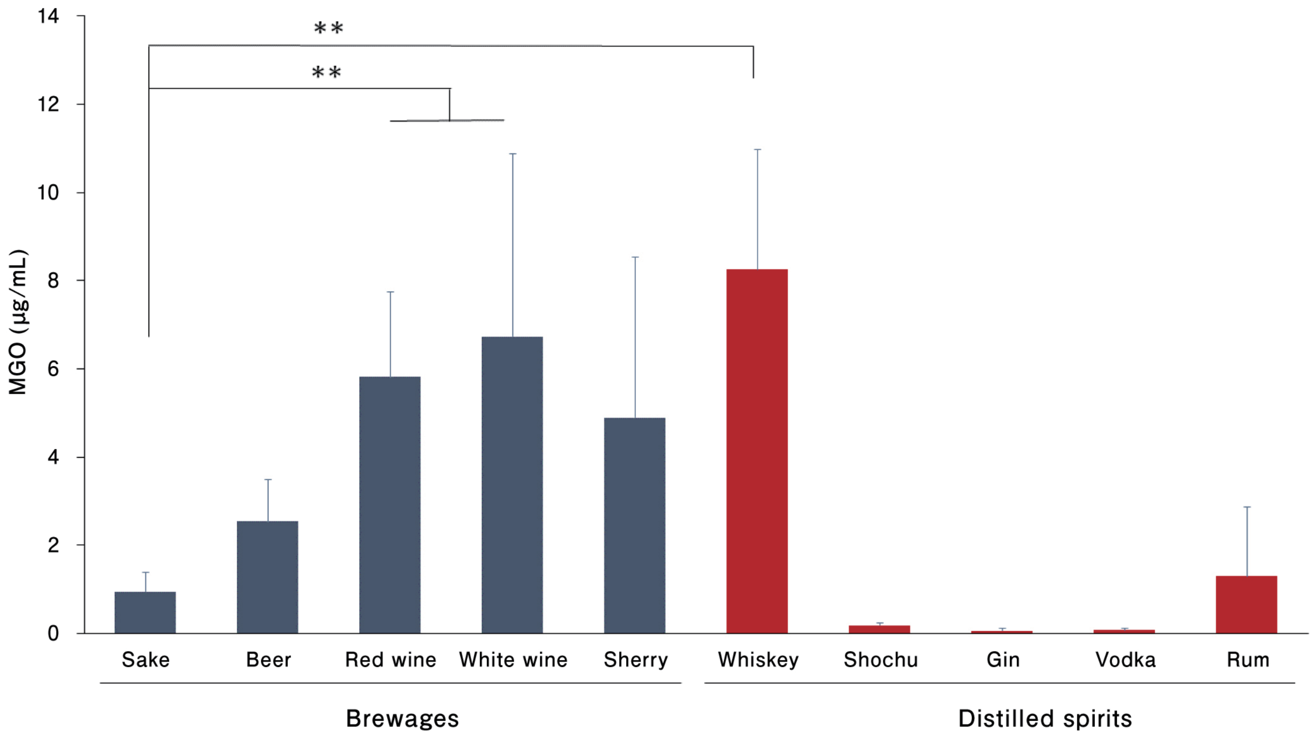

Fig. 7. **Comparison of MGP content by a type of alcoholic beverage.**

Results are expressed as mean ± standard deviation, , ** $p < 0.01$ by Tukey-Kramer test, n = 3 each except sake (n = 6). MGO, methylglyoxal.

lower than that of beer or sherry ($p < 0.001$).

MGO concentrations were higher in whiskey, white wine, and red wine than in sake, shochu, gin, vodka, and rum ($p < 0.05$, ***Fig. 7***). Sherry contained higher MGO than vodka ($p < 0.05$). The MGO value of sake was lower than red wine, white wine, and whiskey ($p < 0.001$).

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 [11, 12]. 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 reaction. The content of aldehyde varies from 30 to 110 µL/mL, which is more than three times different depending on the type of sake [11]. The amount of acetaldehyde contained in sake was the highest for the second-class liquor, according to the old type classification of sake, followed by the first-class liquor and special-grade liquor. The relationship between the production method of sake and the amount of acetaldehyde is as follows: Daiginjo sake (0.26 µL/mL), Old sake (0.475 µL/mL), Ginjo sake (0.96 µL/mL), Junmai sake (1.0 µL/mL), and Hon-jozo sake (1.2 µL/mL) [12]. Acetic acid produced by the oxidation of alcohol reacts with ethanol to produce ethyl acetate. Therefore, Daiginjo sake and Old sake have a unique taste and aroma because they have a small amount of acetaldehyde and are rich in ethyl acetate. Since the brewing method of Daiginjo sake keeps a high alcohol content, the produced ethanol is easily oxidized and becomes rich in ethyl acetate. As for Old sake, the amount of ethyl acetate increases as the alcohol oxidizes due to aging after brewing.

3-DG concentration in sake

It has been reported that the 3-DG content of sake varies from 0.137 mmol/L to 0.407 mmol/L (22.2 to 66.0 µg/mL), a difference of about 3.0 times, and Junmai sake tends to contain a larger amount when compared between the types [11]. In this study, the 3-DG amount of sake samples (n = 6) was the minimum: 16.9 to the maximum: 138.5 µg/mL (0.104 to 0.854 mmol/L), where an 8.2 times difference was observed. In the brewing process of sake, sugar derived from rice starch used as a raw material reacts with 3-DG, thus producing a large amount of furfural. The amount of furfural produced depends on the sugar produced during brewing [13]. The furfural content was higher in the order of Daiginjo sake: 1.5 µL/mL, Ginjo sake: 1.0 µL/mL, Junmai sake 0.6 µL/mL, and Hon-jozo sake 0.4 µL/mL, indicating that the higher the rice polishing rate, the higher the amount of furfural. Furfural is rich also in Old sake (1.0 µL/mL) and is known to give a unique taste and flavor [11,13]. 3-DG was contained in brewages about 57.8 times as much as distilled spirits. Furfural and 3-DG may be the factors that bring out the unique flavor of sake.

GO concentration in sake

GO was detected in all alcoholic beverages except gin and vodka. The difference in GO concentration may be related to the autoxidation of glucose and the aging period in the barrel. Mainly oak (*Quercus crispula*) wood is used for whiskey aging barrels, where the material contains polyphenols such as ellagic acid. Polyphenols have been reported to suppress the formation of α-DCs, *i.e.* 3-DG, GO, and MGO [14], and also to have an antioxidant effect [15], thus probably being involved in the suppression of GO production. One of the characteristics of sake is that the amount of GO is small.

MGO concentration in sake

The MGO content in alcoholic beverages was lower than that of 3-DG, and was recognized in whiskey (8.2 ± 2.9 µg/mL, n = 3), white wine (6.7 ± 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 glycative reaction occurs by heating in the distillation process, and β-damascenone and fural are produced [16, 17]. MGO in whiskey may be an autoxidation product derived from glycated materials during the distillation process. One of the characteristics of sake is that the amount of MGO 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 [18], 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 *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 ~ 130 mg/kg bw/day in Europe [30], ADI 1.0 ~ 1.3 mg/kg bw/day, one-third of that amount is 0.3 ~ 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.

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Conflict of Interest Statement

The authors claim no conflict of interest in this study.

Reference

1) Fujimaki M, Kobayashi N, Kurata T, et al. Reactivities of some carbonyl compounds in Strecker degradation. *Agricultural and Biological Chemistry*. 1968; 32: 46-50.
2) Ogata Y, Kimura M, Kondo Y. Photo-promoted hypochlorite oxidation of α-amino acids: Kinetics and irradiation effect for the Strecker degradation. *Bulletin of the Chemical Society of Japan*. 1981; 54: 2057-2060.
3) Nagai R, Mori T, Yamamoto Y, et al. Significance of advanced glycation end products in aging related disease. *Anti-Aging Med*. 2010; 7: 112-119.
4) Takeuchi M, Takino J, Yamagishi S. Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: A novel therapeutic strategy. *Current Drug Target*. 2010; 11: 1468-1482.
5) Yagi M, Nomoto K, Hori M, et al. The effect of edible purple chrysanthemum extract on advanced glycation end products generation in skin: A randomized controlled clinical trial and *in vitro* study. *Anti-Aging Med*. 2012; 9: 61-74.
6) Yagi M, Shimode A, Hamada U, et al. Evaluation of the anti-glycation effect and the safety of a vinegar beverage containing indigestible dextrin and a mixed herbal extract: A placebo-controlled, double-blind study. *Glycative Stress Res*. 2014; 1: 14-24.
7) Imayasu S, Kawato A. "Functions of brew": Effects of Seishu (Japanese sake) on health and beauty (No. 2). *J Brew Soc Japan*. 1999; 94: 201-208. (in Japanese)
8) Hata Y. Functionality of sake. *Proceedings of Thermal Engineering Conference*. 2007; 1-2. (in Japanese)

9) Tadasue K, Takabe W, Yagi M, et al. Anti-glycative effect of Japanese sake (seishu): Prevention of advanced glycation end product (AGE) formation. *Glycative Stress Res*. 2017; 4: 80-86.
10) Horie Y, Yamagishi Y, Kato S, et al. Alcoholic liver diseases and hepatitis virus C in Japan. *Nihon Arukoru Yakubutsu Igakkai Zasshi*. 2006; 41: 424-430. (in Japaneae)
11) Ikemi M, Saitoh T, Koizumi T, et al. Characteristics of Sake made by different processes (Part 1): Comparison of general and minor components, amino acids, organic acids, and flavor components. *J Brew Soc Japan*. 1981; 76: 831-834. (in Japanese)
12) Ogawa H, Nakajima T, Yoshihara N, et al. Chemical analyses of ingredients in various types of alcoholic drinks of "Sake". *Bull Tokyo Gakugei Univ Div Nat Sci*. 2010; 62: 23-31. (in Japanese)
13) Isogai A, Utsunomiya H, Iwata H. Changes in the concentrations of sotolon and furfural during the maturation of sake. *J Brew Soc Japan*. 2004; 99: 374-380. (in Japanese)
14) Fujieda M, Tanaka T, Suwa Y, et al. Isolation and Structure of Whiskey Polyphenols Produced by Oxidation of Oak Wood Ellagitannins. *J Agric Food Chem*. 2008; 56: 7205-7310.
15) Yamaguchi T, Takabe W, Yagi M, et al. Analysis of anti-glycative components in *Smallanthus sonchifolius* (yacon). *Glycative Stress Res*. 2018; 5: 36-44.
16) Kwok T, Kirkpatrick G, Yusof H, et al. Rapid colorimetric determination of methylglyoxal equivalents for Manuka honey. *J Adv Biol Biotechnol*. 2016; 7: 1-6.
17) Koga K. Whiskey Science: An amazing process forming ripened flavor. Kodansha, Tokyo, 2018. (in Japanese)
18) Johnston M, McBride M, Dahiya D, et al. Antibacterial activity of Manuka honey and its components: An overview. *AIMS Microbiol*. 2018; 4: 655-664.
19) Watanabe K, Rahmasari R, Matsunaga A, et al. Anti-influenza viral effects of honey *in vitro*: Potent high activity of manuka honey. *Arch Med Res*. 2014; 45: 359-365.
20) Malhotra R, Ziahosseini K, Poitelea C, et al. Effect of Manuka honey on eyelid wound healing: A randomized controlled trial. *Ophthalmic Plast Reconstr Surg*. 2017; 33: 268-272.
21) Nayak PA, Nayak UA, Mythili R. Effect of Manuka honey, chlorhexidine gluconate and xylitol on the clinical levels of dental plaque. *Contemp Clin Dent*. 2010; 1: 214-217.
22) Adams CJ, Manley-Harris M, Molan PC. The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr Res*. 2009; 344: 1050-1053.
23) Daglia M, Ferrari D, Collina S, et al. Influence of *in vitro* simulated gastroduodenal digestion on methylglyoxal concentration of Manuka (*Lectospermum scoparium*) honey. *J Agric Food Chem*. 2013; 61: 2140-2145.
24) Wang J, Chang T. Methylglyoxal content in drinking coffee as a cytotoxic factor. *J Food Sci*. 2010; 75: H167-171.
25) Murata-Kamiya N, Kamiya H, Kaji H, et al. Mutations induced by glyoxal and methylglyoxal in mammalian cells. *Nucleic Acids Symp Ser*. 2000; 44: 3-4.
26) Murata M, Mizutani M, Oikawa S, et al. Oxidative DNA damage by hyperglycemia-related aldehydes and its marked enhancement by hydrogen peroxide. *FEBS Lett*. 2003; 554: 138-142.
27) Thornalley PJ. Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems--role in ageing and disease. *Drug Metabol Drug Interact*. 2008; 23: 125-150.
28) Eriksson UJ, Wentzel P, Minhas HS, et al. Teratogenicity of 3-deoxyglucosone and diabetic embryopathy. *Diabetes*. 1998; 47: 1960-1966.
29) Yokoyama A. Risk factors and field cancerization of esophageal squamous cell carcinoma in Japan. *Nihon Shokakibyo Gakkai Zasshi*. 2018; 115: 868-880. (in Japanese)
30) European Commission, Health & Consumer Protection Directorate-General. Scientific Committee on Consumer Products: Opinion on glyoxal. SCCP/0881/05, 2005 https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_023.pdf