



Original Article

Dicarbonyl compounds and sugar contents of Thai commercial beverages

Monthana Weerawatanakorn*

Department of Agro-Industry, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Muang, Phitsanulok, 65000 Thailand.

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Abstract

Glyoxal (GO) and methylglyoxal (MGO), two α -dicarbonyl compounds (RCS) found in humans, cause carbonyl stress following the formation of advanced glycation end products (AGEs). Both are linked to many chronic diseases. Foods, the extrinsic source, could cause the increase of RCS levels in physiological conditions. Previous data showed that high fructose corn syrup is the major source of RCS in beverages. Because of increasing consumption of syrup-sweetened beverages in Thailand, we aimed to find the GO, MGO, sugar contents, and their quantity relationship. We discovered that 70 different types of beverages contained extremely high levels of GO and MGO at maximum level of 333 and 1,208 $\mu\text{g}/\text{ml}$, respectively. All commercial syrup tested contained dicarbonyl contents, and statistics tests showed strong significant correlation between monosaccharide sugar and RCS. The total sugar contents of more than 20 tested was higher than the current daily recommendation for sugar intake to maintain health.

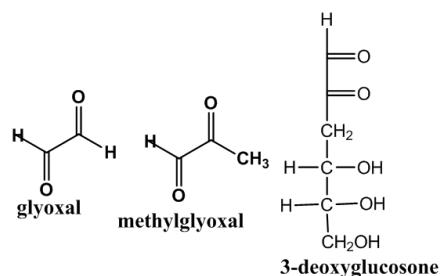
Keywords: methylglyoxal, glyoxal, beverages, syrup, sweetener

1. Introduction

Glyoxal (GO), methylglyoxal (MGO) and 3-deoxyglucosones (3-DOG), whose structures are shown in Figure 1, are reactive carbonyl species (RCS) identified by many clinical studies as a pathogenic factor in microvascular and macrovascular implications and are linked to hyperglycemia and diabetes (Thornalley, 1996; Baynes and Thorpe, 1999; Singh, 2001). With their extremely reactive compounds they are able to accumulate in body fluids and tissues and to modify proteins, DNAs and phospholipids, causing active adducts, in particular the advanced glycation end products (AGEs), leading to carbonyl stress followed by oxidative stress, and cellular dysfunction (Onorato *et al.*, 1998; Baynes *et al.*, 1999). Some AGEs are shown in Figure 2. A series of dicarbonyl compounds can be found both in various food products and the physiological system by the autoxidation of glucose, lipid peroxidation and the elimination of phosphate

in glycolysis (Frye *et al.*, 1998). Out of them, GO and MGO are drawing most attention as major α -dicarbonyl compounds found in humans and are associated with diabetes complications (Nagaraj *et al.*, 2002). Plasma concentration of RCS, especially MGO, is elevated in diabetic patients both with type 1 (insulin-dependent) and type 2 at 2-6-folds higher than those of healthy individuals (Frye *et al.*, 1998; Lo *et al.*, 2008).

In foods, the ∞ -dicarbonyl compounds are generated from heat treatments such as roasting, baking, broiling and frying by three main types of chemical reactions including



* Corresponding author.
Email address: monthanac@nu.ac.th

Figure 1. Glyoxal, methylglyoxal, and 3-deoxyglucosone found in physiological system and food products.

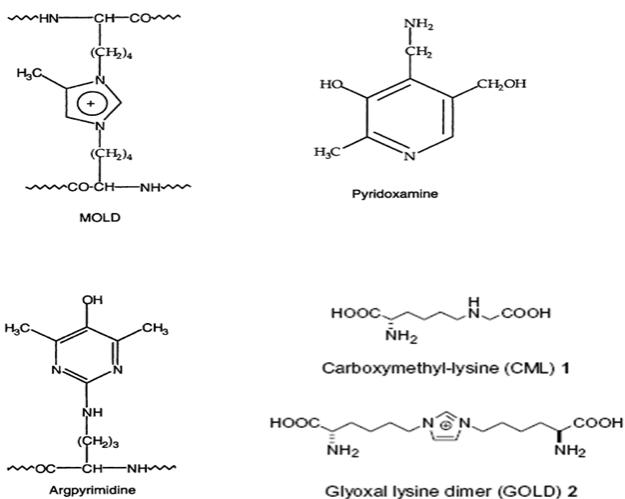


Figure 2. Some of advanced glycation end products (AGEs); MOLD: methylglyoxal-lysine dimer, Pyridoxamine, Argypyrimidine, GOLD: glyoxal lysine dimer, CML: carboxymethyl-lysine (after Ahmed *et al.*, 1997; Frye *et al.*, 1998; Nagaraj *et al.*, 2002).

caramelization, maillard reaction, and lipid oxidation (Homoki-Farkas *et al.*, 1997; Hollnagel and Kroh, 1998). GO and MGO have been detected in foodstuffs including bread, soy sauce, instant tea, roasted coffee, wine, brandy, vinegar, cheese, oil from tuna, cod and salmon, liver, and olive oil heated at 60°C (Rodrigues *et al.*, 1999; Fojika and Shibamoto, 2004). GO and MGO in food and beverages come from sugars generated by the Maillard reaction (fragmentation and retro-aldol reaction) or autoxidation of sugar. Taking MGO as an example, it may be formed by fragmentation of 3-DOG as shown in Figure 3. Therefore, foods are the major extrinsic sources that could cause the increase of RCS levels in normal physiological conditions, yielding excessive AGE production. Recently, Lo *et al.* (2008) found the GO, MGO and 3-DOG in the

commercial beverages, especially carbonated soft drinks, which contain high levels of high fructose corn syrup. Their findings also showed that high fructose corn syrup was identified as a source of these reactive carbonyl compounds. High fructose as well as glucose and fructose syrups are increasingly being used in food and beverage manufacturing in developing countries, such as Thailand, to substitute refined sugar due to economic cost, sensory perception and ease of handling. The principal of syrup processing is conversion of starch to monosaccharide sugars, glucose and fructose.

In Thailand, the manufacturers of commercial non-alcoholic beverages, including carbonated, beauty and energy drinks and fruit and tea beverages are also increasing the use of syrup as a sweetener. The major sweetening ingredient of these non-alcoholic beverages is cane sugar, syrup or a mixture of the two. We suspect the amount of dicarbonyl contents in these commercial beverages and their relationship with types of sugar either mono or disaccharide. We are concerned with the potential hazardous effects of dietary MGO and GO on humans; therefore, this study aims to quantify the amounts of GO and MGO and to evaluate their quantity relationship with different types of sugars in commercial beverages sold in Thailand.

2. Materials and Methods

2.1 Materials

Six groups of commercial beverages (CBs), comprising 70 different beverage types, including two diet carbonated beverages, were used in this study. Each brand was purchased from two different supermarkets (Big C and Tesco-Lotus supermarket). Three types of commercial syrups, 38 (83 °Bx), 38 (85 °Bx) and 85 (75 °Bx) dextrose equivalent syrups were provided by WGC Co., Ltd (Sampran, Nakorn Pathom, Thailand). Unless otherwise indicated, all chemical reagents

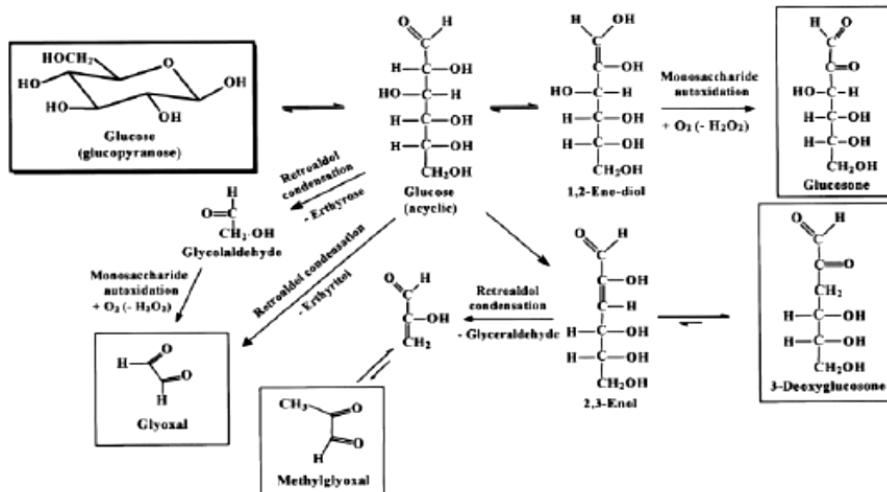


Figure 3. Generation of MG from oxidative degradation of glucose (Nursten, 2005).

were of the highest quality obtainable from Sigma, including MGO (40% aqueous solution). *O*-Phenylenediamine (*O*-PDA) was purchased from Sigma (St. Louis, MO, U.S.A.). Quinoxaline (Q) was purchased from Fluka Chemicals (Milwaukee, WI, U.S.A.). 2-Methyl-quinoxaline (2-MQ; 97%), HPLC grade water, acetonitrile, methanol and ethanol were purchased from Fisher Scientific (Springfield, NJ, U.S.A.).

2.2 Sugar determination

Samples were filtered, degasified, and determined for sugar profiles including fructose, glucose, and sucrose using high-performance liquid chromatography (HPLC) with a refractive index (RI) detector held at 40°C. LC solution software was used for control of the HPLC system. In the mobile phase we used a solution of acetonitrile and water in a ratio of 80/20 previously filtered and degasified. The column used was Innertsil NH₂ (4.6 mm x 250 mm). Working conditions were with a flow rate of 1 ml/min. The analyses were performed in triplicate batches. The standard solutions of different sugars, sucrose, glucose and fructose, were prepared for the quantitative and qualitative determination of sugars in the sample.

2.3 Dicarbonyl compound derivatization

1,2-Dicarbonyl compounds- typically GO and MGO- were analyzed as the corresponding quinoxalines after derivatization with *O*-PDA according to the method described by Mavric, *et al.* (2008), with some slight modifications. Figure 4 shows derivatization and adducts by *O*-PDA. All derivatization processes were the same. 1.0 mL of sonicated sample or standard solutions of the 1,2-dicarbonyl compounds were added with 0.6 mL of 1% w/v solution of *O*-PDA in 0.5 M sodium phosphate buffer, pH 6.0. The mixture was then kept in the dark for 12 hrs at room temperature, then capped and shaken vigorously for 2 min. After membrane filtration (cellulose acetate 0.45 mm), 20 uL of samples were subjected to chromatography analysis.

2.4 HPLC analysis

All experiments were performed using a gradient High Pressure Liquid Chromatography system (Shimadzu HPLC class VP series, Shimadzu Corporation, Kyoto, Japan). Quinoxaline derivatives were separated by InnertSil C18 column (4.6 mm x150), held at 30°C temperature. The class VP software was used for control of the HPLC system and

data processing. Chromatographic conditions for gradient elution were as follows: flow rate, 0.8 mL min⁻¹; volume injected, 20 μ L. UV spectra were recorded at the 312 nm. Separations were performed using a gradient of increasing methanol concentrations in water acidified with 0.15% acetic acid (v/v) as follows: 22 min linear gradient from 20 to 40% methanol, 22 min increasing gradient segment to 100% methanol, followed by 20% methanol for 8 min. The mobile phase composition was taken to the initial condition in 4 min, and the column was equilibrated 10 min before the next injection.

2.5 Statistical methods

All data are given as mean \pm SD. Correlation analyses were examined by Pearson correlation coefficient. Statistically significant differences are defined as P less than 0.05. Data analysis of our sampling was performed by the Windows SPSS (Statistical Package for the Social Sciences) statistical program version 10.

3. Results and Discussion

3.1 Dicarbonyl contents in commercial beverages

There is increasing evidence showing a major pathogenic link between formation of advanced glycation end products (AGEs) and hyperglycemia and diabetes related complications. AGEs, proinflammatory compounds, are formed through nonenzymatic glycation, the reaction between reducing sugar and amino acid compounds also known as the Maillard reaction. Such a reaction generates GO and MGO, a series of very reactive dicarbonyl compounds. The MG concentration in diabetic patients is about 2-6 times as high as in normal people (Frye *et al.*, 1998). AGEs serum circulates are positively associated with endogenous and exogenous sources of ∞ -dicarbonyl compounds (Luevano-Contreras and Chapman-Novakofski, 2010) both GO and MGO, and food is the exogenous source of ∞ -dicarbonyl compounds which may come from sugar. This study aims to determine ∞ -dicarbonyls, sugar contents and their relationships. This is the first report of dicarbonyl contents in food in Thailand. The results of some analysis samples of the 70 different types classified into six groups of beverages are reported in Table 1.

The levels of GO and MGO, the types of sweeteners, and the total amount of sugar content both disaccharide and monosaccharide in some tested products are calculated on

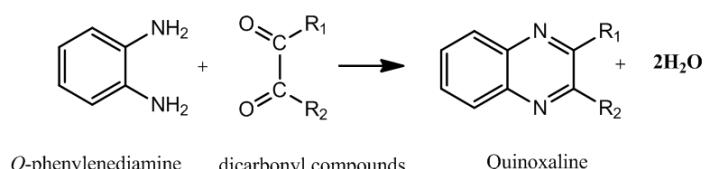


Figure 4. Derivative mechanism used to determine ∞ -dicarbonyl compounds (glyoxal and methylglyoxal).

the basis of experimental results and reported in Table 1. The quantitative method for dicarbonyl determination was performed based on quinoxaline derivative formation. Quinoxaline is a derivative for GO, and 2-methylquinoxaline is a derivative for MGO. HPLC chromatograms of MGO and GO standard and some samples were registered in Figure 5. The peaks for quinoxaline and 2-methylquinoxaline were

confirmed with standard compounds at retention times of 30.6 and 35.2 min, respectively. The results indicated that all samples except the one with sugar substitutes contained α -dicarbonyl compounds (GO and MGO) either one or both.

The results revealed that the levels of reactive carbonyl compounds in commercial drinks were found to be high (Table 1). Although there is the biological detoxification

Table 1. Dicarbonyls levels, sugar contents and types of sweetener presented in different commercial beverages.

Sample	Level of dicarbonyls (μ g/100 ml)		sucrose as sweetener	fructose syrup as sweetener	% disaccharide sugar	% mono saccharide sugar
	GO	MG				
Carbonated beverages						
CB1	112.0 \pm 2.3	ND	+	-	6.0	9.5
CB2	12.7 \pm 0.3	ND	+	-	11.3	2.8
CB3	84.7 \pm 2.2	ND	+	-	1.5	15.1
CB4	108.6 \pm 2.9	ND	+	-	1.1	16.9
Energy drinks						
ED1	61.0 \pm 3.1	ND	+	-	12.9	5.9
ED2	152.6 \pm 2.3	ND	+	-	4.7	11.8
ED3	171.7 \pm 3.2	10.3 \pm 0.5	+	-	8.8	7.8
ED4	236.6 \pm 5.6	9.9 \pm 0.3	+	-	12.3	7.8
Beauty drinks						
BD1	82.2 \pm 0.8	24.0 \pm 0.1	+	+	3.4	6.7
BD2	65.7 \pm 0.8	22.4 \pm 0.2	+	+	1.2	9.1
BD3	47.0 \pm 0.2	23.2 \pm 0.6	-	+	0	8.4
BD4	24.3 \pm 0.5	10.3 \pm 0.1	-	+	0.1	1.9
BD5	71.6 \pm 0.5	15.1 \pm 0.2	+	-	4.6	20.6
Tea beverage						
TB1	311.1 \pm 6.1	253.3 \pm 2.6	+	+	2.6	9.8
TB2	333.4 \pm 4.3	17.9 \pm 0.2	-	+	2.5	8.7
TB3	105.3 \pm 3.2	73.20 \pm 3.5	-	+	1.8	5.2
TB4	116.3 \pm 2.4	9.2 \pm 0.1	+	-	3.9	0.3
TB5	62.0 \pm 2.8	ND	+	+	4.7	1.3
TB6	66.6 \pm 1.5	ND	+	-	4.5	0.5
TB7	87.0 \pm 3.5	86.9 \pm 2.2	+	+	2.9	8.7
TB8	148.3 \pm 1.6	55.7 \pm 2.3	-	+	0	10.5
Fruit beverage						
FB1	2.1 \pm 0.1	ND	+	+	8	5.4
FB2	3.0 \pm 0.3	ND	+	+	4.5	8.4
FB3	313.3 \pm 3.6	75.2 \pm 2.3	+	-	1.0	9.6
FB4	87.3 \pm 3.3	65.5 \pm 1.5	-	+	0.5	12.5
FB5	97.3 \pm 3.2	96.7 \pm 1.9	+	-	4.3	8.6
Flavored beverage						
FFB1	88.4 \pm 2.9	1208 \pm 12.1	+	+	0.1	13.8
FFB2	131.3 \pm 1.3	28.5 \pm 0.6	-	+	0.4	8.6
FFB3	80.7 \pm 2.5	84.5 \pm 1.2	-	+	0.1	11.7
FFB4	24.7 \pm 0.1	16.4 \pm 0.1	+	+	4.8	6.0
FFB5	16.0 \pm 0.1	6.0 \pm 0.2	+	+	4.0	5.8

ND = not detectable

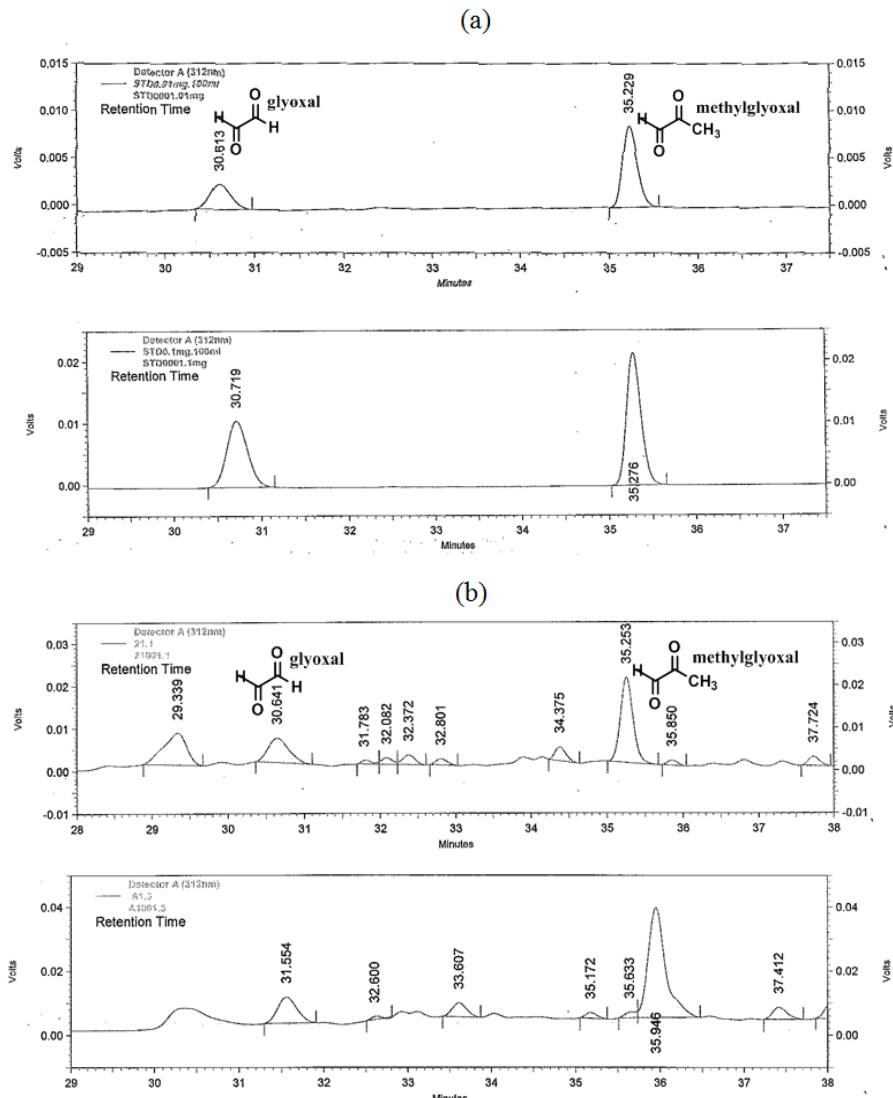


Figure 5. HPLC chromatograms of MGO and GO for (a) standard at 10 and 100 $\mu\text{g}/100 \text{ mL}$ and (b) tested commercial beverages.

system for dicarbonyl compounds (Frye *et al.*, 1998), the range observed is significantly higher than the reported level of plasma MGO, 16–21 $\mu\text{g}/100 \text{ mL}$, in diabetic patients (Odani *et al.*, 1999; Lapolla *et al.*, 2003). The maximum MGO (1,208 $\mu\text{g}/100 \text{ mL}$) and monosaccharide amount (13.8%) were found in lemon flavored beverages (FFB1) with, according to the labels, a mixture of fructose syrup (7%) and sugar cane (5%) as sweeteners. It contained an significant amount of disaccharide sugar (0.1%). The highest amount of GO (333 $\mu\text{g}/100 \text{ mL}$) was detected in a tea beverage (TB2) with 2.5% sucrose and 8.6% monosaccharide (glucose and fructose) and fructose syrup (8.5%) as sweeteners. Statistical results showed that while there was a positive and statistically significant correlation between monosaccharide sugars to GO ($r = 0.20$; $p < 0.05$), we found a negative correlation between disaccharide sugars (sucrose) to MGO with no statistical significance ($r = -0.97$). This suggested that the more disaccha-

ride sugar contents contained in the products, the less MGO is present. Although, it is not clear about this relationship, but it might be due to the fact that GO and MGO are products formed by reducing sugar (monosaccharide sugars) through Maillard reaction. Therefore, the more disaccharide sugars contained, the less dicarbonyl compounds found. We did not find both GO and MGO levels in carbonated beverages with sugar substitutes labeled as “Zero” or “Light” because they contained no mono and disaccharide sugar.

Carbonated beverages contained GO levels ranging between 12.7–112.0 $\mu\text{g}/100 \text{ mL}$, and MGO level was not detected. MGO level in energy drinks were also negligible. Although these two groups of beverages contained relatively high amounts of total sugar compared to the rest of the four groups of beverages, we observed that they had negligible MGO levels, and the sweetener used in these types of beverages was labeled as cane sugar. Tan *et al.* (2008) found

that MGO levels in carbonated beverages in the US were high, ranging between 71.2-267.2 $\mu\text{g}/100 \text{ mL}$, and these beverages used high fructose corn syrup (HFCS) as a sweetener. Later, Lo *et al.* (2008) showed that the source of dicarbonyl compounds in beverages was HFCS. The different result may be attributed to the different sweetener used in carbonated beverages.

Previous research demonstrated that GO and MGO can be effectively trapped by tea polyphenols including theaflavin from black tea and (-)-epigallocatechin gallate (EGCG) from green tea, resulting in reduction of these dicarbonyl compounds (Lo *et al.*, 2006). We found two kinds of fruit drinks in this study, rosella (FB1) and tamarind (FB2) drinks, which showed a very low content of GO and MGO (2.1 and 3.0 $\mu\text{g}/100 \text{ mL}$, respectively) (Table 1) even though they contained high sugar content (13%) in a mixture of cane sugar and fructose syrup as sweeteners, according to the labels. The result also showed that TB5 (green tea) and TB6 (Oolong tea) had low levels of GO and MGO compared with other tea beverages tested. The tea concentration and tea quality of beverages might be an important factor in the lower levels of these two dicarbonyl compounds. It is possible that the phytochemicals in these two kinds of fruits may have the potential to reduce the levels of reactive carbonyl compounds by trapping these dicarbonyl compounds. Further study is needed to verify the phytochemicals and possible mechanisms.

3.2 Dicarbonyl contents in commercial syrup

The sweetener used in carbonated beverages and energy drinks in this study was cane sugar, while the rest of the other groups consisted of a mixture of sugar and syrup. The result showed that fructose syrup, compared to glucose syrup, is the most popular one for beverage manufacturers in Thailand. Out of 70 types of commercial beverages, the highest syrup concentration used was 12% in one of the fruit beverages whereas the maximum sweetener used was 21% in one of the beauty drinks, according to the labels.

Syrup used in Thailand is made from tapioca root or cassava starch while syrup used in the US is made from maize. HFCS primarily has been used in the US as a dietary sweetener. The previous research indicated that HFCS is the major source of dicarbonyl compounds in US beverages. In order to verify the observation of the possible source of dicarbonyl compounds in tested beverages, three kinds of syrup samples, obtained from syrup manufacturers, including 38DE (Dextrose equivalent) (83 °Bx), 38DE (85 °Bx) and 85DE (75 °Bx), were investigated for GO, MGO, types, and levels of sugars. The results are in Table 2. The GO and MGO contents in syrups were concentration dependent and all types of syrup contained high contents of monosaccharide sugar (glucose and fructose) and a very low level of disaccharide (sucrose).

Both GO and MGO were not found in low syrup concentration (4% and 6% syrup). At 10% and 20% syrup concentrations there was no MGO content in both types of 38 DE syrup, but a high content of MGO was observed in 85 DE syrup which increased from 280 to 572 $\mu\text{g}/100 \text{ mL}$. Strong significant positive correlation was found between monosaccharide sugar and dicarbonyl compounds both GO ($r = 0.807$; $p < 0.05$) and MGO ($r = 0.848$; $p < 0.05$). The data suggested that not only the type and the concentrations of syrup, but also the DE value play a role in the contents of GO and MGO. DE means the amount of reduced sugar expressed as glucose, and the higher the DE, the larger the amount of monosaccharides (glucose) and the smaller the amount of dextrins or polysaccharide present.

The results here imply that syrup high in monosaccharides (glucose and fructose) used in factory beverages may be a possible source of dicarbonyl contents. However, the products which contained only cane sugar as sweetener, like carbonated and energy drinks, still contained dicarbonyl compounds. This suggests that, apart from syrup, other possible factors play a role in determining GO and MGO contents in commercial beverages. These include manufacturing processes conditions, such as types of heat treatment, temperature and time. These have an impact on

Table 2. Concentrations of methylglyoxal, glyoxal and sugar content in 10% and 20 % syrup.

% syrup	DE/Brix	Methylglyoxal ($\mu\text{g}/100\text{ml}$)	Glyoxal ($\mu\text{g}/100\text{ml}$)	Sugar content %			
				glucose	fructose	sucrose	total
10	38/83	ND	11.3 \pm 0.6	0.50 \pm 0.01	0.02 \pm 0.03	ND	0.55
	38/85	ND	12.5 \pm 0.8	0.83 \pm 0.09	0.02 \pm 0.01	ND	0.85
	85/75	280.1 \pm 12.2	33.3 \pm 3.9	4.87 \pm 0.32	3.10 \pm 0.01	0.08 \pm 0.01	8.05
20	38/83	ND	65.7 \pm 4.2	9.33 \pm 0.03	0.40 \pm 0.05	ND	9.73
	38/85	ND	49.0 \pm 3.8	8.28 \pm 0.03	0.16 \pm 0.20	ND	8.44
	85/75	572.4 \pm 16.2	131.3 \pm 11.0	8.72 \pm 0.35	5.63 \pm 0.01	0.09 \pm 0.01	14.44

ND = not detectable

sugar content through the transformation of disaccharide to monosaccharide sugar, and GO and MGO were generated by monosaccharide sugar through Maillard reaction and auto-oxidation of sugar (Homoki-Farkas *et al.*, 1997; Hollnagel and Kroh., 1998; Nursten, 2005). Adding syrup to the commercial beverages may be a factor in the increase of reactive dicarbonyl compounds.

3.3 Sugar contents in commercial beverages as a nutrition concern

The nutrition information from the products was also checked. Table 3 shows the amount of total sugar content expressed as teaspoon unit per an individual product serving size, total sugar content stated by the manufacturer on the

Table 3. Total sugar contents of tested some commercial beverages (g/100mL sample).

Beverages (serving size)	^a Total sugar content (Teaspoon/serving size)	Total sugar content on label (%)	^b Percentage difference over from label (%)
Carbonated beverages (325 ml)			
CB1	8	11	6
CB2	10	16	6
CB3	10	15	11
CB4	9	12	49
Energy drinks (100-150 ml)			
ED1	7	18	19
ED2	6	17	21
ED3	8	17	20
ED4	5	18	20
Beauty drinks (180-350 ml)			
BD1	9	9	25
BD2	9	10	20
BD3	8	21	26
BD4	8	11	0
BD5	2	2	0
Tea beverage (325-450 ml)			
TB1	7	6.3	19
TB2	9	8.5	29
TB3	6	6.4	6
TB4	8	6.2	17
TB5	13	9.3	23
TB6	12	10.4	17
TB7	7	8	9
TB8	9	10	15
Fruit beverage (250-325ml)			
FB1	4	7	10
FB2	5	9	12
FB3	7	10	32
FB4	6	10	29
FB5	8	9	42
FB6	6	8	40
Flavored beverage (250-450 ml)			
FFB1	3	5	75
FFB2	5	11	4
FFB3	6	11	0
FFB4	12	13	0
FFB5	5	12	0

^a Calculated based on total sugar from label (sugar 1 tea spoon = 4 g);

^b Percentage of sugar from label over total sugar analyzed by HPLC.

label, and percentage difference between assayed and declared levels of some samples. The current daily recommendation for sugar intake to maintain health should be 4-8 teaspoons depending on the energy needed (1,600-2,400 kcal a day). The 20 products tested had higher sugar content than 8 teaspoons per serving size. The highest total sugar content was 13 teaspoons in one of the tea beverages (TB5) and the lowest content was one teaspoon in beauty drinks (not shown in the table). Beauty drinks in the study refer to the group of beverages claimed by the manufacturers to enhance beauty, mostly related to skin health. The amount of total sugar of this group of products ranged from 1-9 teaspoons per serving size, and three out of nine different samples of this group were higher than the recommended sugar amounts.

Total sugar content per serving size of tea beverages, fruit beverages and fruit-flavored beverages was also high, ranging from 6-13 teaspoons, 4-8 teaspoons, and 3-12 teaspoons, respectively (Table 3, based on HPLC analysis). These products are becoming increasingly popular in Thailand, especially ready-to-drink tea and it is often claimed in product advertisements that they provide specific health benefits either from the tea itself or from the fruits content. There is no doubt about the health benefits of drinking fresh brewed tea, black, green or oolong tea, without sugar, but ready to drink tea with a high sugar content cannot justifiably be called a health drink.

Some of samples tested contained even higher levels of total sugar, calculated from the experimental assay, than declared by the manufacturer on the label as indicated in Table 3. These products contained sugar levels much higher than the amounts stated on the labels, between 4% and 75% higher. A possible explanation for these discrepancies could be that some sweeteners changed from disaccharide to monosaccharide sugar during processing.

4. Conclusion

Our study indicated that 70 different types of commercial beverages the contain high levels of GO and MGO. As we expected, syrups contain GO and MGO and the statistical tests showed strong significant correlation between monosaccharide sugars and dicarbonyl compounds. Phytochemicals in the beverages may have the potential to reduce the levels of reactive carbonyl compounds. Further study is needed to verify the phytochemicals and possible mechanisms. The total sugar levels of commercial beverages in Thailand are high, and that of some health claimed beverages were higher than the current daily recommendation for sugar intake to maintain health.

Acknowledgments

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