Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 1099–1105
Food Chemistry
www.elsevier.com/locate/foodchem

Reactive dicarbonyl compounds and 5-(hydroxymethyl)-2-furfural in carbonated beverages containing high fructose corn syrup

Chih-Yu Lo [a,b,*], Shiming Li [b], Yu Wang [b], Di Tan [b], Min-Hsiung Pan [c], Shengmin Sang [d], Chi-Tang Ho [b,e,*]

[a] Department of Food Science, National Chiayi University, No. 300 Syuefu Road, Chiayi 600, Taiwan
[b] Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA
[c] Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung 811, Taiwan
[d] Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA
[e] Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 106, Taiwan

Received 29 August 2007; received in revised form 10 September 2007; accepted 11 September 2007

Abstract

α-Dicarbonyl compounds, namely glyoxal (GO), methylglyoxal (MGO) and 3-deoxyglucosone (3-DOG), as well as 5-(hydroxymethyl)-2-furfural (5-HMF) were found and measured in carbonated soft drinks (CSD). It was realized that high fructose corn syrup (HFCS) in regular CSDs was the major source of α-dicarbonyl compounds in beverages after comparison of levels in regular and diet CSDs. In two most commonly used HFCS formulas, 42% and 55% HFCS, the highest level of dicarbonyl found was 3-DOG, followed by MGO, and then GO. The stability of dicarbonyls in CSDs containing HFCS and (−)-epigallocatechin gallate (EGCG) which were incubated at 35 and 45 °C was investigated. It was found that EGCG decreased from 1 mg/mL to 0.5 and 0.3 mg/mL for 35 and 45 °C, respectively, in 16 days storage. Moreover, the reactions of EGCG with MGO, 3-DOG and 5-HMF were observed from the comparison of storage CSDs with and without EGCG under acidic conditions.

Keywords: α-Dicarbonyl compounds; Carbonated soft drink; High fructose corn syrup; Glyoxal; Methylglyoxal; 3-Deoxyglucosone; 5-(Hydroxymethyl)-2-furfural

1. Introduction

The enzymatic transformation of glucose to fructose was developed by Marshall and Kooi (1957) and refined by Japanese researchers in the 1970s. Due to its lower price and ease of handling, high fructose corn syrups (HFCS) containing 90%, 55% or 42% fructose were widely used in the food and beverage industry as a liquid sweetener. The demand for HFCS could be reflected from the use of HFCS from 0.5 lb per capita in 1970 to 62.4 lbs in 1997, remaining as high as 59.0 lbs per capita in 2005 (http://www.ers.usda.gov/Data/FoodConsumption/FoodAvailQueriable.aspx#midForm; Putnam & Allshous, 1999). In view of the fact that there is a high consumption of HFCS in beverages nowadays, many nutrition researchers have focused on the possible relationship between beverage consumption and obesity, diabetes or other related chronic diseases (Basciano, Federico, & Adeli, 2005; Bray, Nielson, & Popkin, 2004; Ebbeling et al., 2006; Jürgens et al., 2005; Wharton & Hampl, 2004).

In individuals with hyperglycemia, elevated levels of glucose in the body fluid augment imbalanced physiological

* Corresponding authors. Addresses: Department of Food Science, National Chiayi University, No. 300 Syuefu Road, Chiayi 600, Taiwan. Tel.: +886 5 271 7621; fax: +886 5 271 7596 (C.-Y. Lo). Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA. Tel.: +1 732 932 9611x235; fax: +1 732 932 6776 (C.-T. Ho).
E-mail addresses: chihyulo@mail.ncyu.edu.tw (C.-Y. Lo), ho@aesop.rutgers.edu (C.-T. Ho).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.09.028

metabolisms. Many clinical and experimental studies have identified the formation of glucose degradation and oxidation products known as reactive carbonyl species (RCS), such as glyoxal, methylglyoxal and 3-deoxyglucosone, as a significance clinical factor in microvascular and macrovascular implications (Baynes & Thorpe, 1999; Onorato, Thorpe, & Baynes, 1998; Thornalley et al., 2003). In patients with insulin-dependent and non-insulin-dependent diabetes, the plasma concentration of RCS, in particular methylglyoxal, was 2–6-folds higher than those of healthy individuals (Lapolla et al., 2003; Odani, Shinzato, Matsumoto, Usami, & Maeda, 1999).

Foods are the major extrinsic factor that could cause the increase of RCS in normal physiological conditions. Literature data on the detection and quantification of RCS in foods have not been extensive, as only a few publications reported the levels of α-dicarbonyl compounds in fermented food products, such as wine, brandy, vinegar and cheese. Other than glyoxal and methylglyoxal, diacetyl, penta-2,3-dione and phenylglyoxal were identified in wine (de Revel, Pripis-Nicolau, Barbe, & Bertrand, 2000; Yamaguchi, Ishida, Xuan, Nakamura, & Yoshitake, 1994). Interestingly, α-dicarbonyls have been investigated in honey samples as an indicator of heating processes during manufacturing and storage (Weigel, Opitz, & Henle, 2004).

5-(Hydroxymethyl)-2-furfural (HMF) can be formed in systems which contain monosaccharides in acidic solutions (Murkovic & Pichler, 2006; Nozal, Bernal, Toribio, Jimenez, & Martin, 2001; Teixido, Santos, Puignou, & Galceran, 2006). The potential genotoxic and carcinogenic properties of HMF had been studied and reviewed (Glatt, Schneider, & Liu, 2005; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000). Due to the coexistence of monosaccharides and high acidic situations in most soft drinks, attention has been focused on HMF levels, along with RCS levels, in this study.

A large consumption of sugar-sweetened beverages all over the world is a reason for the current study to screen the possible presence of RCS in commercial carbonated beverages. Because we have previously demonstrated that methylglyoxal can be effectively trapped by polyphenols such as green tea (−)-epigallocatechin gallate (EGCG) (Lo et al., 2006), in the present study, the possible effect of (−)-epigallocatechin gallate (EGCG) on the levels of RCS in carbonated beverages during storage was also investigated.

2. Materials and methods

2.1. Materials

Thirteen different carbonated soft drinks (CSDs) including two diet carbonated beverages were used in this study. Each brand was purchased from three different supermarkets. Honey purchased from a supermarket was a wild flower pure honey product of USA. *o*-Phenylenediamine (*o*-PDA) was purchased from Sigma (St. Louis, MO, USA). It was the derivative reagent used for dicarbonyl compounds. Quinoxaline (Q) was purchased from Fluka Chemicals (Milwalkee, WI, USA). 2-Methylquinoxaline (2-MQ; 97%), 5-(hydroxymethyl)-2-furfural (5-HMF), 2-ethylpyrrole (2-EP; technical grade, 90%) and anhydrous sodium sulfate were purchased from Aldrich (St. Louis, MO, USA). EGCG (100% pure) was a gift from Mitsui Norin (Shizuoka, Japan). Glass threaded vials (14.8 mL; 21 mm × 70 mm; od × H), HPLC grade water, acetonitrile, methanol and ethanol were purchased from Fisher Scientific (Springfield, NJ, USA). Deuterated solvents, including D_2O, were purchased from Norell Company (Landisville, NJ, USA). 2-Methyl-3-(2′,3′-dihydroxypropyl)-quinoxaline (97% purity) was purchased from TRC (Toronto Research Chemicals Inc., Toronto, ON, Canada). TLC was performed on 250 μm thickness, 2–25 μm particle size TLC plates (Sigma–Aldrich, St. Louis, MO, USA). Three samples of HFCS were gifts from two beverage companies. Two were claimed to contain 55% fructose and one was claimed to have 42% fructose.

2.2. Dicarbonyl compound derivatization

The derivatization reaction was done for GO, MGO and 3-DOG. Their corresponding quinoxaline analogues were formed by the reaction of dicarbonyl compounds with *o*-phenylenediamine (*o*-PDA). All derivatization processes were the same. After the samples were added with appropriate amount of *o*-PDA in glass vials, glass vials were capped and shaken vigorously by vortexing for 5 s. The reaction was performed in a 60 °C water bath and 50 rpm for 30 min. After centrifuging at 14 × 1000 rpm (16,000*g*) for 5 min, samples were ready for HPLC analysis.

2.3. 2-Methyl-3-(2′,3′-dihydroxypropyl)-quinoxaline preparation and purification from honey

Ten grams of honey were dissolved with 5 mL *o*-PDA (60 mg/mL). After reacting in a water bath at 60 °C and 50 rpm for 30 min, the solution was applied to a self packed reverse-phase C_{18} column (Borosilicate glass column; i.d. × Length: 1.5 × 30 cm; Sigma Chemical Co., St. Louis, MO, USA). 100% water was used as initial mobile phase. After sugars and unreacted *o*-PDA eluted from the column, the mobile phase was changed to 30% methanol. The fractions with major components were checked by TLC. This whole procedure was repeated to collect enough amounts of major components for further purification.

The major components obtained from the previous step were further dissolved in a mixture of water (1.5 mL) and ethanol (1 mL) and loaded onto the C_{18} reverse-phase preparative (XTerra® MS C18 OBD™, 5 μm) (Waters Corp., Milford, MA, USA) HPLC system. A gradient method was used from 5% acetonitrile −95% water to 20% acetonitrile −80% water in 20 min with a flow rate of 20 mL/min. The fractions were analyzed by LC–ESI-MS. The purified fractions by LC–MS were combined and concentrated or

lyophilized to dryness. The dried compounds were analyzed by MS and NMR and their LC–MS and NMR spectra were the same as the purchased reference. Information on these equipments will be described in later sections.

2.4. Screening of GO, MGO and 3-DOG in carbonated beverages

Thirteen varieties of CSDs (noted as: A, B, C, D, E, F, G, H, I, J, K, AA and BB) used in this study were in aluminum cans. Two of them are diet drinks and they are the same brands with particular brands A and B, respectively (noted as AA and BB). J and K were energy carbonated drinks.

For proper sampling of beverages, 13 varieties of CSDs were purchased from three different local supermarkets on different days. The batch sizes depended on the manufacturers' package. There were 6 or 12 cans in each batch. One can for each batch in a particular brand from each supermarket was sampled. Each sampled beverage was measured in triplicates. The can was uncapped before the experiment and enough amount of beverage was poured into glass vials for sonication for 5 min and 0.9 mL beverage was mixed with 0.1 mL (60 mg/mL) *o*-PDA.

2.5. GO, MGO and 3-DOG in high fructose corn syrup (HFCS)

In order to explore the possible source of dicarbonyl compounds in the beverage, the nutrition information from the products were checked. HFCS is the most suspicious source for these compounds. In the investigation of GO, MGO and 3-DOG in HFCS, 10 g of each HFCS were weighted into 10 mL volumetric flasks and were dissolved to the final concentration as 1 g/mL by *o*-PDA (60 mg/mL). The dicarbonyl compounds were derivatized under conditions similar to those previously described in the section on Dicarbonyl compound derivatization.

2.6. Beverage storage study

Brand A CSD was chosen for further accelerated storage study. A 2×2 factorial experiment was designed in this study. The factors were temperature and EGCG concentration. Temperatures were set at 35 or 45 °C and EGCG's final concentrations in beverages were either 0% or 0.1% (w/v). Four systems were used: (i) beverage without EGCG at 35 °C, (ii) beverage with 0.1% EGCG at 35 °C, (iii) beverage without EGCG at 45 °C, and (iv) beverage with 0.1% EGCG at 45 °C. Four aluminum cans (serving size 12 oz) in the same batch were used in this study and one can was studied in each of the four systems. The EGCG solution (0.03 g/mL) was prepared before the experiment. The beverage was poured into a 500 mL beaker and sonicated for 5 min. The beverage (14.5 mL) was transferred to each vial. Afterwards, 0.5 mL HPLC grade water was added to (i) and (iii), and 0.5 mL, of 0.03 g/mL EGCG was added to (ii) and (iv). Under these circumstances, the headspace volume in a vial was minimal. The samples stored in the oven were kept away from light. Time points of 0, 1, 2, 4, 8, 12 and 16 days were set for EGCG, dicarbonyl compounds (GO, MGO and 3-DOG), and 5-HMF quantifications.

2.7. Sample preparation/derivatization for EGCG, GO, MGO and 3-DOG content analyzes in stability study

For EGCG analysis, samples were centrifuged before HPLC run. For dicarbonyls analyzes, the same procedure was used as described in the section under Dicarbonyl compound derivatization.

2.8. HPLC system

The Dionex UltiMate 3000 LC Modules equipped with a pump (Model: LPG-3400 pump, Sunnyvale, CA), UV–Vis detector (Model: VWD-3400 detector), and an autosampler (Model: WPS-3000 SL) were used. A Luna C_{18} (Phenomenex, Torrance, CA) column (150×4.6 mm i.d., 3 μm particle size) was used for EGCG and quinoxaline derivatives analysis. The column temperature was maintained at 25 °C in column oven (Dionex Model: STH 585). The mobile phase for the HPLC system consisted of HPLC grade water with 0.15% acetic acid (v/v; solvent A) and acetonitrile (solvent B) with a constant flow rate set at 0.8 mL/min. HPLC gradient programs were performed as followings: (i) For EGCG analysis: A curve line number 6 gradient elution was performed as following: Initially, 8% solvent B, and it increased to 40% over 10 min, to 48% over additional 2 min, to 60% over additional 1 min and 5 min for equilibrium. EGCG was detected with a UV wavelength at 280 nm and the injection volume was 15 μL. (ii) For dicarbonyl compounds analysis: The same curve gradient program was performed as in EGCG analysis from 0 to 13 min. After 13 min, solvent B increased to 80% for additional 2.5 min and 5 min for equilibrium. Quinoxaline derivatives were detected with a UV wavelength at 313 nm and the injection volumes were varied and dependent on the concentration of quinoxaline derivatives. The external standard quantification method was applied in this study. Every single peak area for the quantification was laid in the linear range of each standard curves.

2.9. Extraction processes for gas chromatography (GC) analysis

At each specific incubation period at 35 and 45 °C, beverage samples were taken out from oven. The CSD samples were stirred vigorously for 5 s before extraction process. CSD (3 mL) was transferred into a clean vial. Later, an appropriate amount of internal standard was added to 3 mL CSD. 5-HMF in CSD was extracted twice with 3 mL methylene chloride. For each extraction process, samples were stirred vigorously for 5 s. The methylene chloride and CSD were centrifuged for 5 min. The organic

phase was dried over anhydrous sodium sulfate. These extraction samples were concentrated to 0.5 mL final volume under gentle nitrogen gas. The samples were ready for GC injection and quantification.

2.10. Capillary GC/FID

The analyzes of standard volatiles samples were performed with an Agilent Gas Chromatograph (Santa Clara, CA). The Agilent Gas Chromatograph (6850 series) equipped with an Agilent autosampler (7683 series Injector) and a flame ionization detector (FID) was used. Samples were analyzed on a Zebron ZB-5 fused silica capillary column, 30 m × 0.25 mm i.d., film thickness 1.0 μm (Phenomenex, Torrance, CA). The injector temperature was 250 °C and detector temperature was 300 °C. 1 μL sample was injected for analysis and the injector was in splitless mode. The 1.0 mL/min constant carrier gas (helium) flow rate was set. The GC oven temperature was programmed as followed: the initial oven temperature 40 °C held for 0 min and increased to 164 °C at the rate of 4 °C/min held for 0 min. Then the temperature was increased to a final temperature of 280 °C at the rate of 120 °C/min and held for 3 min. The total run time was 34.97 min.

2.11. NMR instrument

NMR spectra were recorded on a Varian 300 Spectrometer (Varian Inc., Palo Alto, CA). With TMS serving as internal standard, ^{1}H NMR was recorded at 300 MHz.

2.12. Liquid chromatography–electrospray ionization mass spectrometry (LC–MS)

An HPLC–MS system was composed of an autosampler injector, an HP1090 system controller, with a variable UV wavelength (190–500 nm) detector, an ELSD (Evaporative Light Scattering Detector) and an ESI-MS detector from Micromass VG Platform II mass analyzer (Micromass, Beverly, MA). ESI-MS conditions were as follows: Acquisition mode, ESI-positive; mass scan range, 100–800 amu; scan rate, 0.4 s; cone voltage, 25 V; source temperature: 150 °C; probe temperature: 550 °C. Analytical HPLC conditions on HPLC–MS: Column: Chromeabond WR C_{18} (ES Industries, West Berlin, NJ), 3 μm, 120 Å; length and OD: 30 × 3.2 mm; injection volume: 15 μL; flow rate: 2 mL/min; run time: 3 min. Mobile phase consisted of acetonitrile and H_2O with 0.05% TFA, typical gradient of 10–90% acetonitrile and the gradient varied.

2.13. Statistical analysis

Data were expressed as means ± standard deviation (SD) and represent three independent analyzes. Statistical significance was examined using Student's *t*-test comparison between the means. A p value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. GO, MGO and 3-DOG in carbonated beverages

Table 1 lists the concentrations of GO, MGO and 3-DOG in 13 brands of carbonated soft drink (CSD) from three different local supermarkets. The baseline separation of quinoxaline derivatives by HPLC can be achieved in a 15 min gradient run. The peaks for quinoxaline and 2-methylquinoxaline were confirmed with standard compounds at retention times of 11.35 and 12.67 min, respectively. The peak with retention time at 7.81 min was first checked with a LC–MS to have a molecular weight at 234 corresponding to the quinoxaline derivative of

Table 1
The concentrations of GO, MGO and 3-DOG in 13 brands carbonated soft drinks from supermarket A, B and C

Brand	Supermarket								
	A			B			C		
	GO	MGO	3-DOG	GO	MGO	3-DOG	GO	MGO	3-DOG
A	50.8 ± 1.6	88.3 ± 7.2	3488.9 ± 50.6	39.9 ± 8.1	83.6 ± 6.1	3346.7 ± 12.3	28.9 ± 3.1	78.2 ± 6.7	3197.0 ± 70.2
B	24.8 ± 1.5	92.5 ± 8.4	3338.4 ± 89.9	25.1 ± 0.8	89.2 ± 3.4	2875.4 ± 21.6	25.5 ± 0.9	87.3 ± 8.8	2636.6 ± 47.1
C	27.3 ± 0.7	54.6 ± 0.6	2647.1 ± 0.7	11.2 ± 0.7	46.5 ± 3.1	2401.8 ± 41.7	7.2 ± 3.5	60.0 ± 10.7	2638.5 ± 73.1
D	25.2 ± 3.8	48.5 ± 2.1	2813.7 ± 60.3	12.3 ± 1.7	23.6 ± 1.5	1351.6 ± 81.2	8.5 ± 0.4	41.8 ± 1.3	2733.7 ± 14.5
E	15.8 ± 6.5	41.1 ± 4.8	1779.6 ± 9.4	10.8 ± 2.2	31.6 ± 2.6	1566.4 ± 50.0	9.5 ± 3.0	31.0 ± 1.6	1549.1 ± 30.9
F	27.8 ± 0.8	42.5 ± 2.0	2229.8 ± 9.5	11.4 ± 1.2	38.5 ± 0.8	2373.6 ± 99.0	12.9 ± 1.2	29.2 ± 1.2	1647.5 ± 54.5
G	173.4 ± 2.8	62.2 ± 3.3	2460.6 ± 39.5	68.8 ± 2.9	57.8 ± 1.0	2835.1 ± 33.5	62.9 ± 2.1	62.1 ± 1.5	2788.9 ± 142.8
H	41.9 ± 2.2	50.1 ± 6.1	2796.6 ± 42.3	21.0± 1.5	50.2 ± 0.5	2765.6 ± 33.1	22.0 ± 1.3	27.7 ± 0.2	1286.7 ± 2.8
I	34.6 ± 0.9	23.5 ± 0.6	1677.4 ± 27.1	18.3 ± 0.9	26.2 ± 1.1	1374.5 ± 32.4	20.5 ± 0.5	26.9 ± 0.8	1334.8 ± 6.5
J	104.6 ± 14.4	59.7 ± 4.8	1339.5 ± 35.8	40.8 ± 8.5	43.2 ± 4.9	1656.2 ± 99.4	33.9 ± 1.9	33.0 ± 3.2	1697.5 ± 13.5
K	38.4 ± 1.9	139.5 ± 2.1	1532.5 ± 7.7	20.8 ± 4.8	104.2 ± 5.9	978.2 ± 50.8	32.7 ± 11.0	111.2 ± 6.0	1016.0 ± 7.4
AA	2.7 ± 0.7	7.2 ± 1.6	19.2 ± 3.3	2.6 ± 0.5	7.6 ± 0.7	10.6 ± 0.7	2.3 ± 0.9	9.3 ± 2.6	13.8 ± 4.1
BB	2.0 ± 0.2	7.1 ± 1.9	ND[a]	2.4 ± 0.4	8.2 ± 1.2	ND[a]	2.0 ± 0.5	31.5 ± 6.2	ND[a]

Concentration unit: μg/100 mL; values are mean ± standard deviation, $n = 3$.
[a] ND = not detectable.

3-DOG, 2-methyl-3-(2′,3′-dihydroxypropyl)-quinoxaline. 2-Methyl-3-(2′,3′-dihydroxypropyl)-quinoxaline was purified and identified from a honey derivatized sample as previously reported (Weigel et al., 2004). Its retention time was 7.81 min, the same as the peak resulting from CSD. The standard curve for 2-methyl-3-(2′,3′-dihydroxypropyl)-quinoxaline was established with a reference compound.

From the results in Table 1, it is obvious that CSD contained significantly high levels of GO, MGO, especially 3-DOG. This is the first report of the presence of reactive dicarbonyl compounds in CSD. AA and BB were both diet drinks and contained aspartame as the sweetener. Less than 20 μg/100 mL or undetectable amounts of 3-DOG were observed in AA and BB. These samples also had low levels of GO and MGO (2.0–9.3 μg/100 mL), except one beverage BB had 31.5 μg/100 mL of MGO from the supermarket C. These dicarbonyl compounds in diet carbonated beverages may originate from caramel or the contaminated natural and artificial flavors. However, beverages A, B and all other brands contained high amounts of HFCS. Their contents of GO, MGO and 3-DOG were in the range of 15.8–104.6, 23.5–139.5 and 978.2–3488.9 μg/100 mL, respectively. In energy drink K, the highest amount of MGO, above 100 μg/100 mL was present.

3.2. GO, MGO and 3-DOG in high fructose corn syrup (HFCS)

In order to verify the observations of dicarbonyls in CSD and their possible sources in drink, 3 HFCS samples were obtained from local beverage companies. In Table 2, high contents of GO, MGO and 3-DOG were observed. The highest level of 3-DOG was in 42% HFCS. More than 7 mg was observed in 100 mL of 42% HFCS. It was more than twice higher than 55% HFCS. However, the concentrations of GO and MGO vary significantly from different sources of HFCS. Their manufacturing processes and storage conditions should play a major role in the observed variations.

3.3. Beverage stability study during storage

After the discovery of high contents of dicarbonyls in CSD which contained HFCS, the storage study was further investigated. Brand A beverage was chosen in this study. A 2×2 factorial experiment was conducted. Besides the temperature factor, EGCG is a natural product used in the stability study. EGCG quantification in EGCG-containing CSDs at the specific time points was monitored from direct HPLC injection of samples without derivatization of dicarbonyl compounds. Since the UV wavelength at 280 nm has higher sensitivity than 313 nm for EGCG. EGCG was detected with UV wavelength at 280 nm and quinoxalines of GO, MGO and 3-DOG was detected with UV wavelength at 313 nm. Not only the concentrations of dicarbonyl compounds but also the levels of 5-MHF were monitored in this storage study. It is known that 5-HMF can be formed from 3-DOG through β-elimination of water under acidic conditions. The identification and quantification of 5-HMF was accomplished by the reference standard. From the results shown in Fig. 1, EGCG reacted with reactive components in CSD under acidic conditions at 35 and 45 °C during storage. The rate of reaction at 45 °C was higher than that at 35 °C. The difference of decreased percentage was 3–4% before tow days. After four days, the difference of decreased percentage increased to 12–18%. The highest difference, 18%, occurred on day eight and the lowest, 12%, occurred on day four. Only half of EGCG was left on day 16 for 35 °C and one-third of EGCG was left on day 16 for 45 °C under storage.

The changes of GO, MGO and 3-DOG levels in beverage with and without the addition of EGCG during storage are shown in Figs. 2–4. In Fig. 2, the original GO levels were among 20–35 μg/100 mL. The highest concentration (37 μg/100 mL) occurred at 35 °C after 16 days. At 45 °C, GO was in a steady level through 16 days study regardless of whether EGCG was added or not. Nevertheless, the 35 °C and EGCG model showed decreased GO level after 16 days of storage, and the highest relative decrease was at eight days. It was the only system that showed a significant decrease in GO level.

In Fig. 3, the concentrations of MGO among four bottled beverages were between 64 and 76 μg/100 mL initially. The generation of MGO under elevated temperatures was a

Table 2
The concentrations of GO, MGO and 3-DOG in HFCS samples

	GO	MGO	3-DOG
#1	18.1 ± 0.8	385.1 ± 7.5	2713.1 ± 89.0
#2	50.8 ± 0.2	112.4 ± 0.1	3332.2 ± 34.2
#3	32.7 ± 1.5	366.9 ± 19.5	7449.0 ± 429.5

Concentration unit: μg/100 mL; values are mean ± standard deviation, $n = 3$; #1 and #2 are 55% HFCS samples and #3 is a 42% HFCS sample.

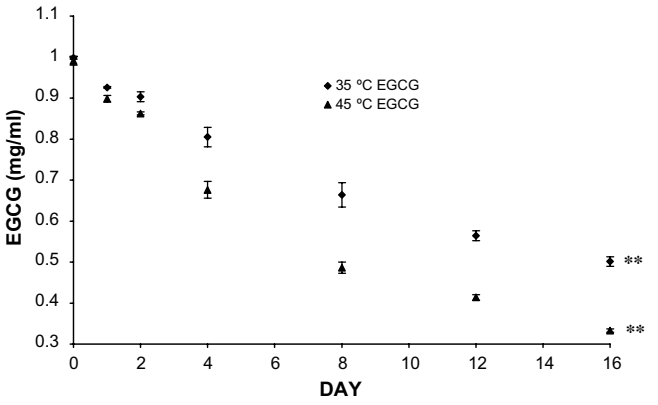

Fig. 1. Concentration of EGCG during storage of brand A beverage from 0 to 16 days at 35 °C (♦) and 45 °C (▲). Each experiment was independently performed in triplicate and expressed as mean ± SD. Asterisks denoted a statistically significant decrease compared with its corresponding initial concentrations (day 0; $^{**}p < 0.01$).

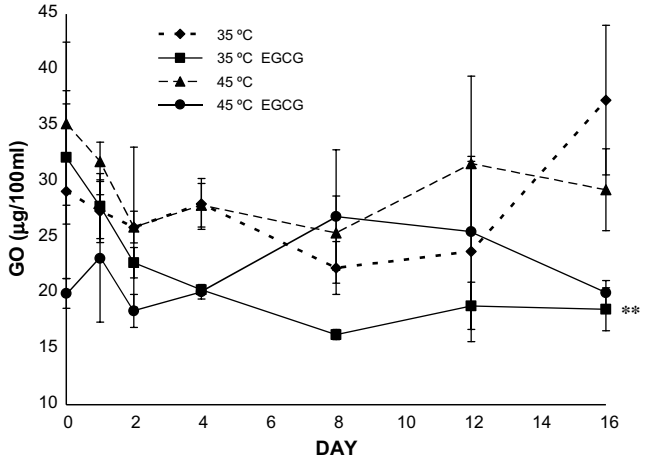

Fig. 2. Glyoxal (GO) changes in brand A CSD samples. ♦: 35 °C, without EGCG; ■: 35 °C, with 0.1% EGCG; ▲: 45 °C, without EGCG; ●: 45 °C, with 0.1% EGCG. Each experiment was expressed as mean ± SD. Asterisks denoted a statistically significant decrease compared with its corresponding initial concentrations (day 0; $^{**}p < 0.01$ and $n = 3$).

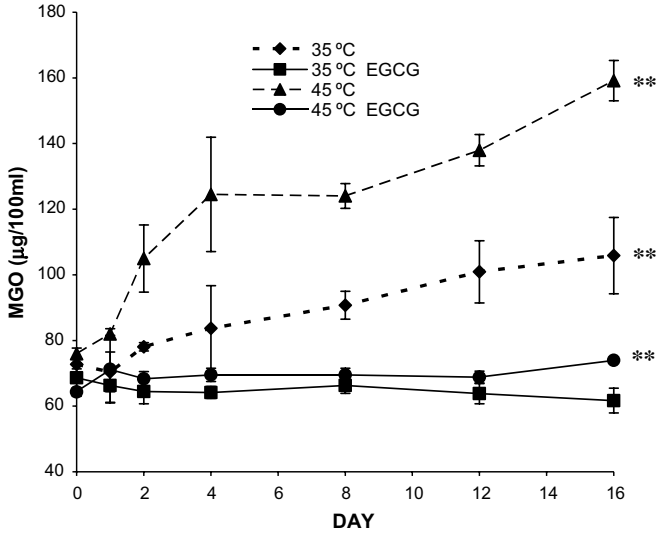

Fig. 3. Methylglyoxal (MGO) changes in brand A CSD samples. ♦: 35 °C, without EGCG; ■: 35 °C, with 0.1% EGCG; ▲: 45 °C, without EGCG; ●: 45 °C, with 0.1% EGCG. Each experiment was expressed as mean ± SD. Asterisks denoted a statistically significant decrease compared with its corresponding initial concentrations (day 0; $^{**}p < 0.01$ and $n = 3$).

static kinetic. According to the observations, MGO concentration for 35 °C after 16 days of storage increased 46% and MGO concentration was doubled after 16 days of storage for the 45 C° sample. However, MGO levels did not increase significantly when EGCG was added to the samples which were incubated at 35 °C and only 16% MGO increase was found in the 45 °C system. It implied that the same adduct reaction between EGCG and MGO had occurred under acidic conditions as we had observed previously (Lo et al., 2006). From the constant concentrations of MGO in the samples with EGCG throughout the

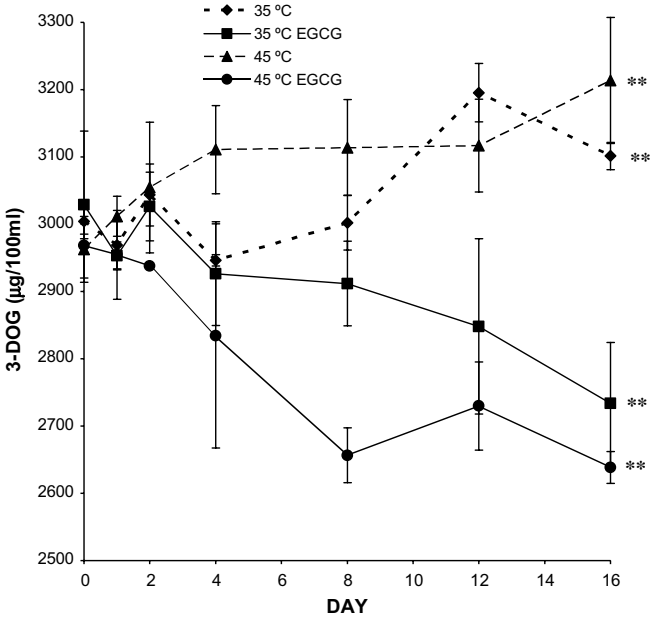

Fig. 4. 3-Deoxyglucosone (3-DOG) changes in brand A CSD samples. ♦: 35 °C, without EGCG; ■: 35 °C, with 0.1% EGCG; ▲: 45 °C, without EGCG; ●: 45 °C, with 0.1% EGCG. Each experiment was expressed as mean ± SD. Asterisks denoted a statistically significant decrease compared with its corresponding initial concentrations (day 0; $^{**}p < 0.01$ and $n = 3$).

entire storage study, it may be concluded that the rates of MGO generation and MGO trapping are similar.

From Fig. 4, the range of 3-DOG varied from 2968 to 3029 μg/100 mL for samples at 0 day. For 45 °C storage, 3-DOG increased from 2968 to 3214 μg/100 mL. The first stage of increase occurred in the first four days of incubation and the next stage occurred after 12 days. Its increase could be due to the autoxidation of glucose (Thornalley, Langborg, & Minhas, 1999). For 35 °C storage, 3-DOG increased from 3004 μg/100 mL in 0 day sample to 3108 μg/100 mL in 16 days sample. In contrast, 3-DOG decreased at both 35 and 45 °C when the beverages contained EGCG. This again confirms our previous observation that dicarbonyl compounds could efficiently react with catechins.

Due to the unexpected high amount of 3-DOG in HFCS containing beverages, 5-HMF was chosen as an indicator for the inappropriate storage condition study. From the GC analysis in Fig. 5, 11–12 mg/L 5-HMF was measured in the beverages initially. Under 16 days storage at 45 and 35 °C, 5-HMF concentrations were more than doubled and increased 48%, respectively. It is interesting to note that the concentration of 5-HMF decreased constantly in the presence of EGCG during storage.

In this study, the levels of reactive carbonyl compounds in commercial carbonated soft drinks were found to be astonishingly high. Taking methylglyoxal as an example, the range observed was 23.5–139.5 μg/100 mL which is significantly higher than the reported level of methylglyoxal, 16–21 μg/100 mL in diabetic patients (Lapolla et al.,

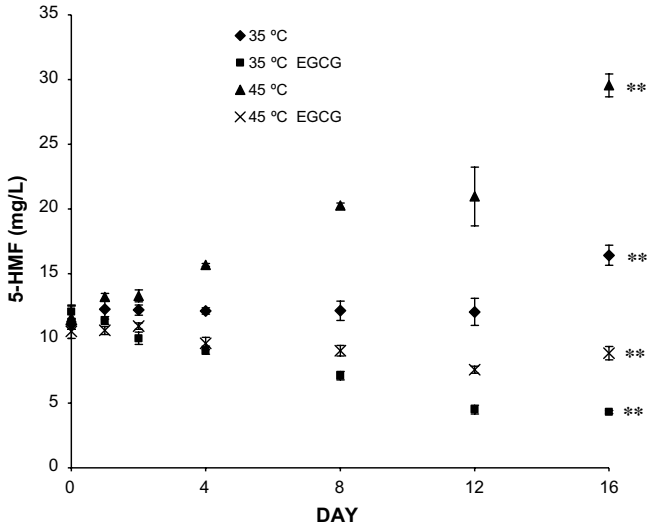

Fig. 5. Correlation of 5-HMF in brand A CSD samples. ♦: 35 °C, without EGCG; ■: 35 °C, with 0.1% EGCG; ▲: 45 °C, without EGCG; ●: 45 °C, with 0.1% EGCG. Each experiment was expressed as mean ± SD. Asterisks denoted a statistically significant decrease compared with its corresponding initial concentrations (day 0; $^{**}p < 0.01$ and $n = 3$).

2003; Odani et al., 1999). High fructose corn syrup was identified as a source of these reactive carbonyl compounds in CSDs. Furthermore, EGCG and possibly other polyphenols may have the potential to reduce the levels of reactive carbonyl compounds in beverages.

References

Basciano, H., Federico, L., & Adeli, K. (2005). Fructose, insulin resistance, and metabolic dyslipidemia. *Nutrition and Metabolism (London), 2*(1), 5.

Baynes, J. W., & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes, 48*(1), 1–9.

Bray, G. A., Nielson, S. J., & Popkin, B. M. (2004). Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity [see comment] [erratum appears in Am J Clin Nutr 2004 Oct;80(4): 1090]. *Diabetes, 79*(4), 537–543.

de Revel, G., Pripis-Nicolau, L., Barbe, J-C., & Bertrand, A. (2000). The detection of alpha-dicarbonyl compounds in wine by formation of quinoxaline derivatives. *Journal of the Science of Food and Agriculture, 80*(1), 102–108.

Ebbeling, C. B., Feldman, H. A., Osganian, S. K., Chomitz, V. R., Ellenbogen, S. J., & Ludwig, D. S. (2006). Effects of decreasing sugar-sweetened beverage consumption on body weight in adolescents: a randomized, controlled pilot study. *Pediatrics, 117*(3), 673–680.

Glatt, H., Schneider, H., & Liu, Y. (2005). V79-hCYP2E1-hSULT1A1, a cell line for the sensitive detection of genotoxic effects induced by carbohydrate pyrolysis products and other food-borne chemicals. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 580*(1–2), 41–52.

<http://www.ers.usda.gov/Data/FoodConsumption/FoodAvailQueriable.aspx#midForm>.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., & Eisenbrand, G. (2000). 5-Hydroxymethylfurfural: Assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food and Chemical Toxicology, 38*(9), 801–809.

Jürgens, H., Haass, W., Castañeda, T. R., Schürmann, A., Koebnick, C., Dombrowski, F., et al. (2005). Consuming fructose-sweetened beverages increases body adiposity in mice. *Obesity Research, 13*(7), 1146–1156.

Lapolla, A., Flamini, R., Dalla Vedova, A., Senesi, A., Reitano, R., Fedele, D., et al. (2003). Glyoxal and methylglyoxal levels in diabetic patients: Quantitative determination by a new GC/MS method. *Clinical Chemistry and Laboratory Medicine: CCLM/FESCC, 41*(9), 1166–1173.

Lo, C. Y., Li, S., Tan, D., Pan, M. H., Sang, S., & Ho, C. T. (2006). Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. *Molecular Nutrition and Food Research, 50*(12), 1118–1128.

Marshall, R. O., & Kooi, E. R. (1957). Enzymatic conversion of D-glucose to D-fructose. *Science, 125*(3249), 648–649.

Murkovic, M., & Pichler, N. (2006). Analysis of 5-hydroxymethylfurfural in coffee, dried fruits and urine. *Molecular Nutrition and Food Research, 50*(9), 842–846.

Nozal, M. J., Bernal, J. L., Toribio, L., Jimenez, J. J., & Martin, M. T. (2001). High-performance liquid chromatographic determination of methyl anthranilate, hydroxymethylfurfural and related compounds in honey. *Journal of Chromatography A, 917*(1–2), 95–103.

Odani, H., Shinzato, T., Matsumoto, Y., Usami, J., & Maeda, K. (1999). Increase in three alpha, beta-dicarbonyl compound levels in human uremic plasma: Specific in vivo determination of intermediates in advanced Maillard reaction. *Biochemical and Biophysical Research Communications, 256*(1), 89–93.

Onorato, J. M., Thorpe, S. R., & Baynes, J. W. (1998). Immunohistochemical and ELISA assays for biomarkers of oxidative stress in aging and disease. *Annals New York Academy of Sciences, 854*, 277–290.

Putnam, J. J., & Allshous, J. E. (1999). Food consumption, prices, and expenditures, 1970–97. In: *US department of agriculture economic research service statistical bulletin no. 965, April 1999* (p. 196). Washington, DC: US Government Printing Office.

Teixido, E., Santos, F. J., Puignou, L., & Galceran, M. T. (2006). Analysis of 5-hydroxymethylfurfural in foods by gas chromatography–mass spectrometry. *Journal of Chromatography A, 1135*(1), 85–90.

Thornalley, P. J., Langborg, A., & Minhas, H. S. (1999). Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochemical Journal, 344*(Pt 1), 109–116.

Thornalley, P. J., Battah, S., Ahmed, N., Karachalias, N., Agalou, S., Babaei-Jadidi, R., et al. (2003). Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry. *Biochemical Journal, 375*(Pt 3), 581–592.

Weigel, K., Opitz, T., & Henle, T. (2004). Studies on the occurrence and formation of 1,2-dicarbonyls in honey. *European Food Research and Technology, 218*(2), 147–151.

Wharton, C. M., & Hampl, J. S. (2004). Beverage consumption and risk of obesity among native Americans in Arizona. *Nutrition Reviews, 62*(4), 153–159.

Yamaguchi, M., Ishida, J., Xuan, Z. X., Nakamura, A., & Yoshitake, T. (1994). Determination of glyoxal, methylglyoxal, diacethyl and 2,3-pentanedione in fermented foods by high-performance liquid chromatography with fluorescence detection. *Journal of Liquid Chromatography, 17*, 203–211.