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Analysis of Sugar Degradation Products with α-Dicarbonyl Structure in Carbonated Soft Drinks by UHPLC-DAD-MS/MS

Sabrina Gensberger,[†] Marcus A. Glomb,[‡] and Monika Pischetsrieder*[,†]

[†]Department of Chemistry and Pharmacy, Food Chemistry, Emil Fischer Center, University of Erlangen-Nuremberg, Schuhstrasse 19, 91052 Erlangen, Germany

[‡]Institute of Chemistry, Food Chemistry and Environmental Chemistry, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Strasse 2, 06120 Halle Germany

ABSTRACT: Sugar-sweetened carbonated soft drinks (CSDs) are broadly consumed worldwide. The added sugar, particularly high-fructose corn syrup (HFCS), can be an important source of sugar degradation products, such as α-dicarbonyl compounds. This study recorded the α-dicarbonyl profile in CSDs by ultrahigh-performance liquid chromatography with hyphenated diode array−tandem mass spectrometry after derivatization with *o*-phenylenediamine. Thus, 3-deoxy-D-*erythro*-hexos-2-ulose (3-DG), D-*lyxo*-hexos-2-ulose (glucosone), 3-deoxy-D-*threo*-hexos-2-ulose (3-DGal), 1-deoxy-D-*erythro*-hexos-2,3-diulose (1-DG), 3,4-dideoxyglucosone-3-ene (3,4-DGE), methylglyoxal, and glyoxal were identified as major α-dicarbonyls and, with the exception of glyoxal, quantified (recovery rates, 85.6−103.1%; RSD, 0.8−3.6%). Total α-dicarbonyl concentration in 25 tested commercial products ranged between 0.3 and 116 μg/mL and was significantly higher in HFCS-sweetened CSDs compared to CSDs sweetened with HFCS and sucrose or with sucrose alone. Predominant was 3-DG (≤87 μg/mL) followed by glucosone (≤21 μg/mL), 3-DGal (≤7.7 μg/mL), 1-DG (≤2.8 μg/mL), methylglyoxal (≤0.62 μg/mL), and 3,4-DGE (≤0.45 μg/mL).

KEYWORDS: *sugar degradation products, α-dicarbonyl compounds (α-DCs), carbonated soft drinks (CSD), high-fructose corn syrup (HFCS), o-phenylenediamine (OPD)*

INTRODUCTION

Carbonated soft drinks (CSDs) are broadly consumed worldwide. In the United States, for example, per capita food availability of CSDs is about 175 L per year.[1] The majority (about three-fourths) of these soft drinks are sugar-sweetened. In the U.S. market, high-fructose corn syrup (HFCS), an ingredient mainly consisting of glucose and fructose, is added to nearly all sugared CSDs.[2] In Europe, however, only a few sodas contain a combination of HFCS and sucrose; most products are sweetened with only sucrose. Recently, it has been demonstrated that HFCS contains considerable amounts of sugar degradation products. Thus, the α-dicarbonyl compounds (α-DCs) 3-deoxy-D-*erythro*-hexos-2-ulose (3-deoxyglucosone, 3-DG), 3-deoxy-D-*threo*-hexos-2-ulose (3-deoxygalactosone, 3-DGal), 1-deoxy-D-*erythro*-hexos-2,3-diulose (1-deoxyglucosone, 1-DG), D-*lyxo*-hexos-2-ulose (glucosone), 3,4-dideoxyglucosone-3-ene (3,4-DGE), glyoxal (GO), and methylglyoxal (MGO) were detected in concentrations up to 1130 μg/mL in HFCS.[3,4] Most likely, α-DCs are formed during the industrial production of HFCS, which is obtained by hydrolysis of starch and subsequent partial enzymatic isomerization of glucose to fructose. Thermal treatment leads to dehydration, oxidation, tautomerization, and C−C cleavage of glucose and fructose, resulting in the different α-DC structures. The α-DC contaminants in the HFCS sweetener are most likely carried through the production process of the CSD and are still present in the consumer product. Thus, 3-DG, GO, and MGO have been identified and quantified in HFCS-sweetened CSDs.[4,5] Additionally, it can be expected that the reducing sugars glucose and fructose are further degraded during the processing or storage of CSDs, increasing the α-DC concentration in the final product. α-DCs are chemically and biologically reactive structures. It is well established that α-DCs can easily modify proteins, thus leading to the formation of advanced glycation end-products.[6,7] Following reaction mechanisms similar to protein glycation, α-DCs can also readily modify DNA in vitro, leading to the formation of DNA-advanced glycation end-products.[8−11] The glycating activity of α-DCs is much higher compared to that of their sugar educts, so α-DCs are important glycation precursors despite their lower concentration.[12,13] Apart from the glycating activity, α-DCs can also have cytotoxic effects or modulate cell signaling in vitro.[14−16] Facts on the bioavailability and physiological consequences of α-DCs in food, however, are still largely unknown.

Thus, it is important to monitor the composition and concentration of α-DCs in food products. The present study used targeted screening to identify the predominant α-DCs in various CSDs. Furthermore, a method was developed and applied to quantify the major α-DCs in commercial beverages, and the relationship between α-DC content and the type of sweetener was investigated.

MATERIALS AND METHODS

Chemicals. All chemical products were of the highest quality available and were obtained from Acros (Geel, Belgium), AppliChem (Darmstadt, Germany), Fluka (Steinheim, Germany), or Sigma

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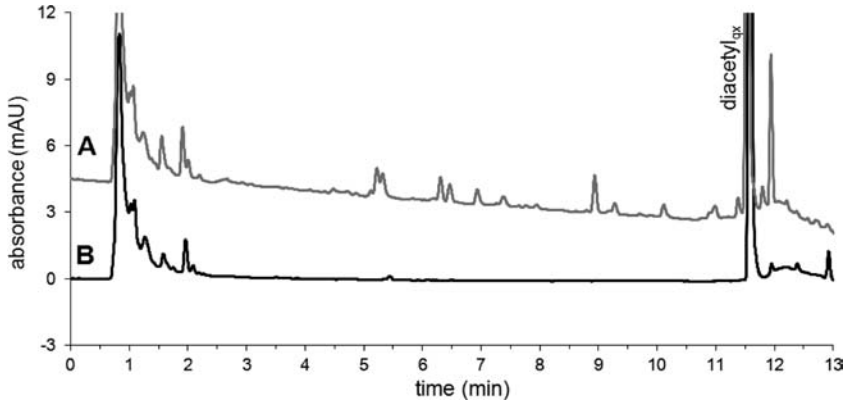


Figure 1. UHPLC-DAD chromatograms of a carbonated soft drink sample spiked with diacetylquinoxaline before (A) and after (B) solid-phase extraction without precolumn derivatization recorded at 316 nm.

(Steinheim, Germany) unless otherwise noted. For all experiments, high-purity water was taken from a Synergi-185 labwater system (Millipore, Schwalbach, Germany).

3-DG (purity > 95%) was purchased from Chemos (Regenstauf, Germany). 3-DGal and the quinoxaline derivative of 1-DG were synthesized as described before.[3,17] Glucosone and 3,4-DGE were synthesized according to the method of Mittelmaier et al.[18,19]

CSD Samples. In this study, 25 different retail CSD samples were screened for α-DCs: 14 of the soft drinks were sweetened with HFCS, 5 with a combination of HFCS and sucrose, and 6 with sucrose. Samples were packed in either cans or plastic bottles. One CSD had been filled in a glass bottle. Aliquots of 40 mL were taken from all samples and degassed for about 10 min by sonication.

Solid-Phase Extraction (SPE). A Strata C18-E cartridge (200 mg/3 mL; Phenomenex, Aschaffenburg, Germany) was first conditioned with 4 mL of methanol and then with 8 mL of water. An aliquot of 2.0 mL of CSD was applied to the cartridge. The sample passage was collected, and remaining polar substances were eluted with 3 mL of water (flow rate ca. 1.5 mL/min). The eluate was freeze-dried, and the residue was dissolved in 2.0 mL of water and used for further analysis. Each CSD was purified by SPE in triplicate.

Derivatization Procedure. Before derivatization, all HFCS-containing samples were diluted with water (1:1). All other purified CSDs were used without any further dilution. Precolumn derivatization of α-DCs was carried out with *o*-phenylenediamine (OPD) in the presence of diethylenetriaminepentaacetic acid (DTPA). Therefore, a derivatization solution of 2% OPD and 11 mM DTPA in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES; 1 M, pH 7.0) was prepared. Ten microliters of the internal standard (diacetylquinoxaline, 50 μg/mL in water) and 45 μL of the derivatization reagent were added to 45 μL of the beverages. The samples were mixed thoroughly and immediately filtered through a syringe filter (PVDF membrane, 0.22 μm, Roth, Karlsruhe, Germany). The reaction mixture was incubated in amber glass vials with limited volume inserts at room temperature and was fed into the liquid chromatography system within 2−4 h after the addition of the derivatization reagent.

Control samples were analyzed without precolumn derivatization. The controls were treated exactly as the CSD samples except that the derivatization solution was replaced by water. Every sample was prepared in triplicate.

Ultrahigh-Performance Liquid Chromatography with Hyphenated Diode Array−Tandem Mass Spectrometry (UHPLC-DAD-MS/MS). A 10 μL aliquot of the derivatized samples was analyzed by UHPLC-DAD-MS/MS using a BEH phenyl column (Waters, Eschborn, Germany) and an ammonium formate/methanol gradient as reported previously.[3] Signals were detected with DAD, which allows recording full UV spectra of the signals. The products were quantified at 335 nm for 3,4-DGE and at 316 nm for all other α-DCs.

For MS/MS experiments an API 4000 QTrap mass spectrometer (AB Sciex, Foster City, CA, USA) with an electrospray ionization (ESI) source (Applied Biosystems, Foster City, CA, USA) was coupled to the UHPLC equipment as described before.[3]

Validation of the Method and Quantification of α-DCs. A calibration curve was prepared for each α-dicarbonyl by plotting the quotient of analyte peak area and diacetylquinoxaline peak area against the concentration of the corresponding α-DC. A nine-point calibration was prepared for 3-DG and 3-DGal each, an eight-point model for glucosone as well as for 3,4-DGE, and a seven-point-calibration curve for MGO. The concentration of 1-DG was evaluated via the calibration model for 3-DG. Each calibration level was measured four times in water. Linear regression of each calibration curve was verified by a minimally acceptable correlation coefficient of 0.9990 and additionally tested by Mandel's fitting test. This statistical tool investigates whether a calibration problem is fitted better by a linear or by a quadratic function.[20] Repeatability of the method was calculated from six independent measurements of one CSD sample and expressed as the coefficient of variation.

The limit of detection (LOD) and limit of quantification (LOQ) of the method have been determined previously as follows: glucosone, 12.4 ng/mL (LOD) and 41.3 ng/mL (LOQ); 3-DG, 11.1 and 36.9 ng/mL; 3-DGal, 14.8 and 49.2 ng/mL; 3,4-DGE, 7.0 and 23.3 ng/mL; MGO, 12.2 and 40.2 ng/mL.[3]

Because the exact composition of the CSD matrix was unknown and no α-DC-free CSD matrix was available, the recovery rate was analyzed in two different ways. First, three different concentrations of α-DCs in water were analyzed including SPE. For each sugar degradation product and each concentration level the recovery rate was calculated as (α-DC concentration/added α-DC concentration) × 100%.

To evaluate the matrix influence, the recovery rate was determined by analyzing one CSD sample before SPE and the same sample after SPE application. For this purpose, a CSD sample without caffeine or other interferents was chosen. The sample was treated as described above in triplicate; in parallel, an aliquot of the CSD was diluted one-to-one with water and derivatized as reported three times without prior purification. The recovery rate was calculated as follows: (amount analyte after SPE/amount analyte before SPE) × 100%.

To analyze the de novo formation of α-DCs in a CSD matrix, CSD samples were reacted with OPD as described above. These samples were applied to the UHPLC-DAD system after various time periods. Data were analyzed by plotting the quotient of analyte peak area and internal standard peak area against the derivatization time.

RESULTS

The aim of this study was to identify the major α-DCs in commercial CSDs by targeted screening and to quantify these sugar degradation products in various CSD samples. For this purpose, α-DCs were selectively converted into their quinoxaline derivatives by OPD. This method has been successfully

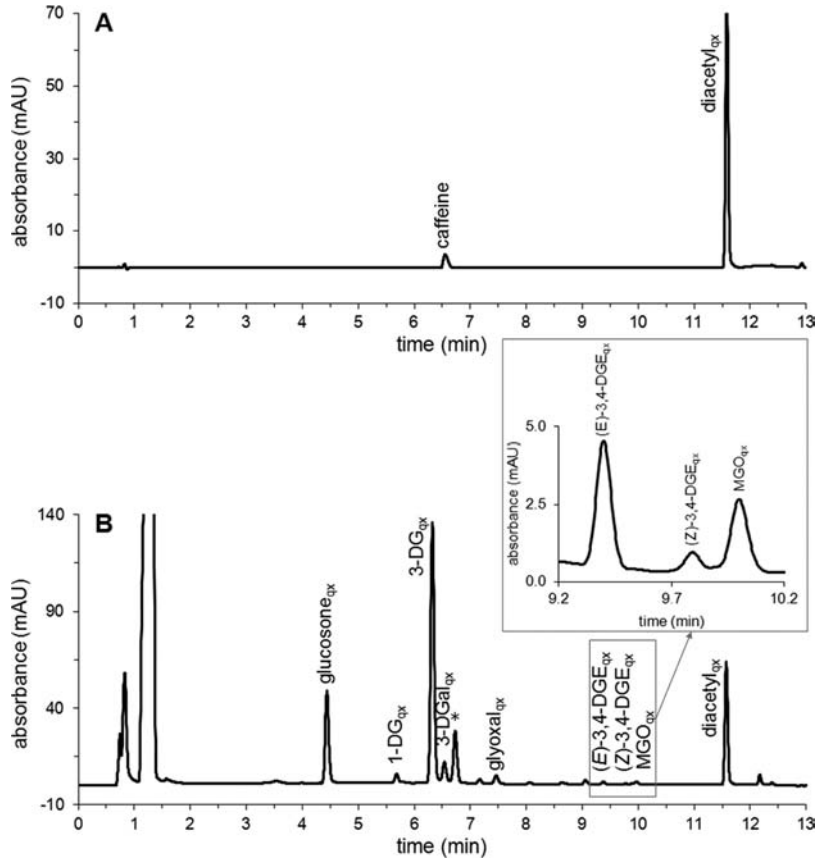


Figure 2. UHPLC-DAD chromatograms of a caffeine solution with diacetylquinoxaline (A) and a carbonated soft drink sample after derivatization of α-dicarbonyl compounds by *o*-phenylenediamine (B) recorded at 316 nm. 3,4-Dideoxyglucosone-3-ene (3,4-DGE) was analyzed at 335 nm (see inset). An asterisk indicates that the respective product is no quinoxaline derivative.

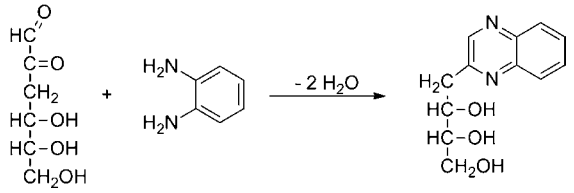
applied before to analyze α-DCs in model mixtures and various other matrices.[3,18,21−23]

Purification of CSD Samples by SPE. To test whether matrix components of the different soft drinks could interfere with the chromatographic separation and UV detection of α-DC quinoxalines, several CSD samples were analyzed without the derivatization step. In these experiments, several signals were detected (Figure 1A). Those signals showed a low response but were dispersed over the whole chromatogram. Thus, reliable quantification of the quinoxaline derivatives of α-DCs required sample purification before analysis. Especially caffeine, a very common ingredient of colas and other soft drinks, partially coeluted with 3-DG and 3-DGal and considerably interfered with the analysis (Figure 2A). Using reversed phase SPE columns, the interferents could be effectively separated from the polar α-DCs (Figure 1B).

α-DC Profiling of CSDs. For the analysis by UHPLC-DAD, α-DCs are efficiently trapped by OPD to form stable quinoxaline derivatives (Scheme 1). Quinoxalines show a very characteristic UV spectrum with a maximum at 316 nm. α,β-Unsaturated quinoxalines, such as 3,4-DGE$_{qx}$, display a bathochromic shift of the UV maximum resulting in an absorbance maximum at 335 nm. The samples were derivatized in the presence of the chelating agent DTPA to suppress metal ion catalyzed oxidation of the sugars.

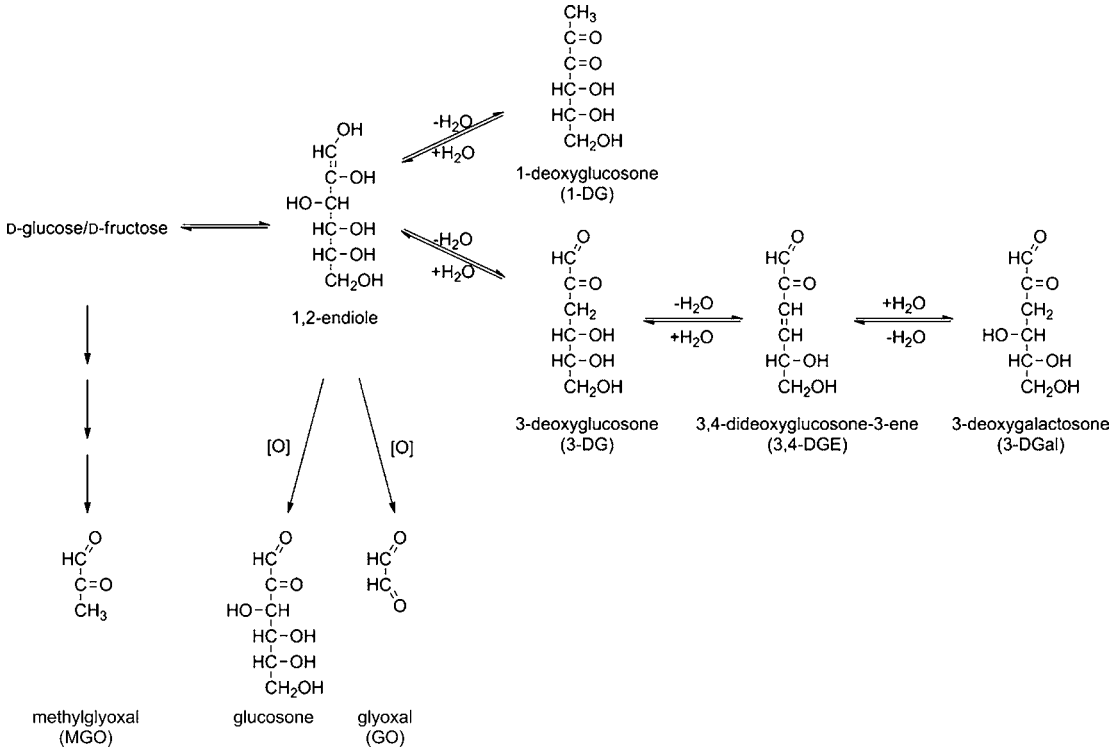
After SPE purification and derivatization, the samples were separated by UHPLC. Figure 2B shows a chromatogram recorded at 316 nm of a typical CSD sample. To enhance sensitivity, 3,4-DGE$_{qx}$ was analyzed at 335 nm (see inset in Figure 2B). All peaks showing the characteristic UV absorbance spectrum of quinoxalines or α,β-unsaturated quinoxalines were assigned to the respective α-DCs by comparing the retention times to those of independently synthesized α-dicarbonyl reference compounds. For unequivocal peak identification, mass spectra as well as product ion scans were recorded for all putative α-DCs by UHPLC-MS/MS (data not shown). Thus, glucosone, 1-DG, 3-DG, 3-DGal, GO, (*E*)-3,4-DGE, (*Z*)-3,4-DGE, and MGO were identified in the CSD samples (Figure 2B; Scheme 2).

Scheme 1. Derivatization of α-Dicarbonyl Compounds with *o*-Phenylenediamine To Yield the Corresponding Quinoxaline Derivatives, Exemplified for 3-Deoxyglucosone (3-DG)



Validation of the Method. Prior to quantification, the linearity of the calibration, repeatability, recovery, and LOD/LOQ were determined. Results of the calibration for each α-dicarbonyl are displayed in Table 1. 1-DG was quantified using the calibration curve of 3-DG. All calibration models show a very good linearity with $R^2 \geq 0.9990$ and comply with the test of linearity according to Mandel ($p > 0.05$). The repeatability of

Scheme 2. α-Dicarbonyl Compounds Identified in Various Commercial Carbonated Soft Drinks[a]



[a]Similar sugar degradation pathways have been observed in other matrices.[3,24,29]

Table 1. Calibration Models for the Analysis of α-Dicarbonyl Compounds by UHPLC-DAD after Derivatization with *o*-Phenylenediamine

analyte	linear regression	R^{2a}	concn range (μg/mL)
glucosone	$0.0903x - 0.0177$	0.9997	0.1–45.2
3-deoxyglucosone	$0.0777x - 0.0044$	0.9999	0.1–45.4
3-deoxygalactosone	$0.0787x + 0.0045$	1.0000	0.1–45.1
3,4-dideoxyglucosone-3-ene	$0.4826x + 0.0635$	0.9991	0.1–15.0
methylglyoxal	$0.2114x - 0.0208$	0.9991	0.1–14.9

[a]Correlation coefficient.

this method is outlined in Table 2 and expressed as the relative standard deviation (RSD) of six independent measurements of one CSD sample. The RSD for all analytes was <5%. The recovery rates of α-DCs in water ranged from 85.6 to 103.1% (Table 3). Analyzing a CSD sample without any interferents prior to SPE and after SPE application led to recovery rates between 90.6 and 95.8% (Table 4).

Table 2. Repeatability of the Method Calculated from Six Independent Measurements of a Carbonated Soft Drink Sample (CSD5; for Analyte Concentrations see Table 5) and Expressed as the Relative Standard Deviation (RSD)

analyte	RSD (%)
glucosone	1.1
1-deoxyglucosone	3.6
3-deoxyglucosone	0.8
3-deoxygalactosone	3.2
3,4-dideoxyglucosone-3-ene	3.0
methylglyoxal	2.9

Table 3. Recovery Rates of Different Concentrations of α-Dicarbonyl Compounds in Water

dicarbonyl				
glucosone	c^a (μg/mL)	44.8	10.1	1.5
	recovery (%)	90.8	92.2	103.1
3-deoxyglucosone	c (μg/mL)	44.1	10.0	0.9
	recovery (%)	90.4	91.3	95.6
3-deoxygalactosone	c (μg/mL)	44.5	10.0	1.1
	recovery (%)	94.1	92.8	90.3
3,4-dideoxyglucoson-3-ene	c (μg/mL)	12.0	8.9	0.8
	recovery (%)	93.7	88.9	85.6
methylglyoxal	c (μg/mL)	14.4	8.0	1.1
	recovery (%)	93.5	91.0	102.5

[a]Concentration.

It has been shown before that GO can be formed de novo in a highly concentrated sugar matrix during derivatization, even in the presence of the metal chelator DTPA. In contrast, de novo formation of other α-DCs has not been detected under these conditions.[3] Therefore, it was investigated if de novo formation also occurred during the derivatization of CSD samples. In CSDs sweetened with HFCS, HFCS/sucrose, or sucrose, the GO_{qx} concentration strongly increased with increasing derivatization time between 0 and 15 h (Figure 3). Increasing GO_{qx} concentration during derivatization may

Table 4. Influence of Solid-Phase Extraction (SPE) on the Recovery of α-Dicarbonyl Compounds (α-DCs) in Carbonated Soft Drinks (CSDs)[a]

analyte	recovery (%)
glucosone	92.8
1-deoxyglucosone	95.8
3-deoxyglucosone	92.0
3-deoxygalactosone	90.6
3,4-dideoxyglucosone-3-ene	93.7
methylglyoxal	92.4

[a]Concentrations of α-DCs in an interferent-free CSD sample were determined before and after SPE (n = 3).

indicate a slow derivatization rate or de novo formation of GO. In the case of a slow derivatization rate, a strong increase would be expected at the beginning reaching a plateau after prolonged derivatization.[24] However, in the current experiments, the formation rates increased with increasing derivatization time in two samples, and a plateau was reached in none of the samples. Thus, these reaction kinetics indicate de novo formation of GO during derivatization rather than slow derivatization. Therefore, GO had to be excluded from quantitative analysis. However, because GO was already detectable in HFCS-containing samples when the analysis was carried out directly after the application of the derivatization agent (t = 0 h), it was concluded that a low concentration of GO was already present in the CSD samples.

Quantification of Six α-DCs in Commercial CSDs. After validation, the method was applied to 25 different commercial CSD samples, and the concentrations of glucosone, 1-DG, 3-DG, 3-DGal, 3,4-DGE, and MGO were analyzed (Table 5). In the case of 3,4-DGE, the sum of both isomers is given, because the *E*-isomer is converted into the *Z*-isomer during derivatization, whereas the sum of both isomers remains constant.[24] The total amount of α-DCs in CSDs sweetened solely by HFCS ranged between 54 and 116 μg/mL (median = 72 μg/mL), whereas CSD sweetened with a combination of HFCS and sucrose contained 1−57 μg/mL (median = 3 μg/mL). In the group of sucrose-sweetened CSDs, total amounts of α-DCs ranged between <1 and 15 μg/mL (median = 2 μg/mL, Figure 4). Highly significant differences between the α-DCs concentration of group 1 (CSD with HFCS) and group 2 (CSD with HFCS/sucrose) as well as between groups 1 and 3 (CSD with sucrose) were determined (one-way ANOVA with post hoc Fisher's LSD test; $p < 0.001$, ***; $p < 0.01$, **; $p < 0.05$, *; Figure 4), whereas differences between groups 2 and 3 were not significant (p = 0.34). In all samples, the most abundant α-DC was 3-DG, followed by glucosone and 3-DGal. Lower concentrations were found for 1-DG, 3,4-DGE, and MGO (Table 5).

Furthermore, the influence of the packaging material on the α-DC concentration was considered. Formation or degradation of α-DCs during storage may be influenced by metal ions from the can or by oxygen diffusion through the package material. Three of the sample products were available in plastic as well as metal containers (CSD2/CSD3, CSD7/CSD8, and CSD22/CSD23). For the two HFCS-sweetened products, similar total α-DC concentrations were detected in the canned and in the plastic-bottled product ($\Delta \leq 2.5$ μg/mL, which is in the range of the SD). In the sucrose-sweetened product, the total α-DC concentration in the plastic-bottled product was considerably higher than in the canned product (Table 5). However, further studies are required to determine if this difference was indeed related to the packaging material.

DISCUSSION

Chemically and biologically reactive α-DCs can be formed by the degradation of sugars during processing. Therefore, α-DCs have been analyzed in different foods, such as honey, wine, beer, coffee, or milk, as well as in medicinal products.[21,25−28]

Very recently, the six major α-DCs have been identified in HFCS.[3−5] Due to the frequent use of HFCS as sweetener in CSDs and their high consumption rate, these beverages could be a major extrinsic source of α-DCs. Therefore, the present study investigated the α-DC profile as well as the concentration of the major α-DCs. Thus, the presence of 3-DG, GO, MGO, and 3-DGal, which had been detected before in CSDs, could be verified.[4,5,29] Additionally, glucosone, 1-DG, and 3,4-DGE were identified for the first time in commercial CSD samples. The α-DC profile in CSDs was very similar to the profile in HFCS, indicating carry-over of α-DCs from the sweetener raw product to the final consumer product. However, further studies are

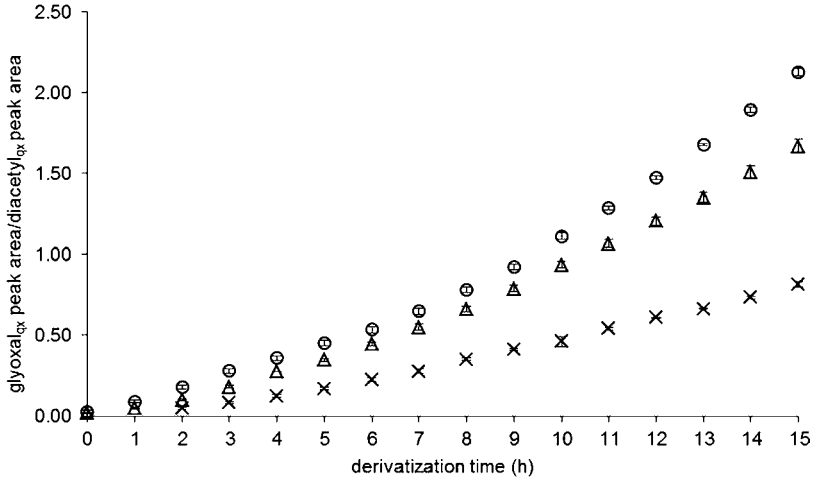


Figure 3. Effect of derivatization time on the glyoxal$_{qx}$ concentration in three different kinds of carbonated soft drink (CSD), containing high-fructose corn syrup (HFCS) (○), HFCS and sucrose (△), or sucrose (×). The mean ± standard deviation of three independent experiments is shown.

Table 5. Concentrations of Six Different α-Dicarbonyl Compounds in 25 Commercial Soft Drink (CSD) Samples (Value ± Standard Deviation; n = 3)

sample	packing	sweetener	glucosone (μg/mL)	1-DG[a] (μg/mL)	3-DG[b] (μg/mL)	3-DGal[c] (μg/mL)	3,4-DGE[d] (μg/mL)	MGO[e] (μg/mL)	sum[f] (μg/mL)
CSD1	can	HFCS	6.41 ± 0.21	1.05 ± 0.04	48.14 ± 1.61	4.88 ± 0.34	0.14 ± 0.01	nd[g]	60.63 ± 1.14
CSD2	plastic	HFCS	12.96 ± 0.64	0.72 ± 0.05	61.21 ± 2.66	6.17 ± 0.13	0.45 ± 0.04	0.41 ± 0.03	81.91 ± 1.45
CSD3	can	HFCS	10.93 ± 0.61	0.68 ± 0.07	69.37 ± 0.95	3.06 ± 0.04	0.13 ± 0.01	0.27 ± 0.01	84.44 ± 1.06
CSD4	can	HFCS	15.63 ± 0.48	0.98 ± 0.03	51.40 ± 1.96	3.11 ± 0.20	0.18 ± 0.01	nd	71.32 ± 0.94
CSD5	can	HFCS	19.09 ± 0.25	2.49 ± 0.15	67.74 ± 2.03	6.39 ± 0.26	0.35 ± 0.01	0.63 ± 0.01	96.70 ± 0.87
CSD6	can	HFCS	11.85 ± 0.21	1.09 ± 0.03	52.05 ± 0.89	3.21 ± 0.23	0.11 ± 0.01	0.23 ± 0.004	68.54 ± 0.85
CSD7	can	HFCS	6.26 ± 0.38	1.98 ± 0.12	42.58 ± 2.46	4.35 ± 0.30	0.30 ± 0.03	0.46 ± 0.02	55.93 ± 2.44
CSD8	plastic	HFCS	5.28 ± 0.25	1.95 ± 0.09	41.54 ± 1.73	4.42 ± 0.20	0.27 ± 0.02	0.64 ± 0.03	54.11 ± 1.17
CSD9	can	HFCS	12.68 ± 0.35	1.07 ± 0.04	47.43 ± 1.28	3.66 ± 0.11	0.32 ± 0.02	0.44 ± 0.02	65.59 ± 1.12
CSD10	can	HFCS	17.16 ± 1.36	2.76 ± 0.04	87.26 ± 1.24	7.68 ± 0.62	0.43 ± 0.01	0.45 ± 0.01	115.74 ± 1.55
CSD11	can	HFCS	6.71 ± 0.19	1.84 ± 0.06	46.38 ± 1.76	4.16 ± 0.17	0.33 ± 0.02	0.62 ± 0.02	60.03 ± 0.05
CSD12	can	HFCS	21.04 ± 0.38	2.29 ± 0.16	66.65 ± 0.97	5.42 ± 0.39	0.20 ± 0.01	0.51 ± 0.01	96.10 ± 0.48
CSD13	can	HFCS	9.52 ± 0.28	1.21 ± 0.04	67.01 ± 2.15	5.31 ± 0.46	0.24 ± 0.02	nq[h]	83.32 ± 0.97
CSD14	can	HFCS	10.31 ± 0.27	1.10 ± 0.02	55.92 ± 2.54	5.64 ± 0.14	0.09 ± 0.01	0.27 ± 0.01	73.33 ± 2.43
CSD15	plastic	HFCS + sucrose	3.65 ± 0.14	0.27 ± 0.01	12.10 ± 0.43	0.66 ± 0.03	nq	nq	16.74 ± 0.19
CSD16	plastic	HFCS + sucrose	0.26 ± 0.01	nd	0.16 ± 0.01	nd	nq	nq	0.51 ± 0.02
CSD17	glass	HFCS + sucrose	14.14 ± 0.23	1.02 ± 0.02	39.99 ± 0.50	2.05 ± 0.14	0.14 ± 0.01	nd	57.34 ± 0.58
CSD18	plastic	HFCS + sucrose	nq	nd	0.56 ± 0.02	nq	nq	nq	0.72 ± 0.02
CSD19	plastic	HFCS + sucrose	1.04 ± 0.02	0.53 ± 0.01	1.06 ± 0.02	nq	nq	nd	2.72 ± 0.03
CSD20	plastic	sucrose	2.34 ± 0.18	0.86 ± 0.07	9.01 ± 0.64	2.63 ± 0.20	0.17 ± 0.01	0.21 ± 0.01	15.21 ± 0.98
CSD21	plastic	sucrose	0.34 ± 0.01	nd	0.50 ± 0.05	nd	nd	0.14 ± 0.01	1.01 ± 0.12
CSD22	can	sucrose	0.59 ± 0.05	nd	1.05 ± 0.04	nd	nq	nq	1.72 ± 0.06
CSD23	plastic	sucrose	1.36 ± 0.12	1.56 ± 0.14	6.51 ± 0.63	1.91 ± 0.17	0.93 ± 0.04	0.21 ± 0.02	12.49 ± 1.01
CSD24	plastic	sucrose	0.90 ± 0.06	nd	0.54 ± 0.04	nd	nq	0.08 ± 0.01	1.57 ± 0.16
CSD25	plastic	sucrose	0.25 ± 0.01	nd	nq	nd	nd	nd	0.33 ± 0.01

[a]1-Deoxyglucosone, [b]3-Deoxyglucosone. [c]3-Deoxygalactosone. [d]3,4-Dideoxyglucosone-3-ene. [e]Methylglyoxal. [f]The sums include values of LOD and LOQ. [g]Not detectable. [h]Not quantifiable.

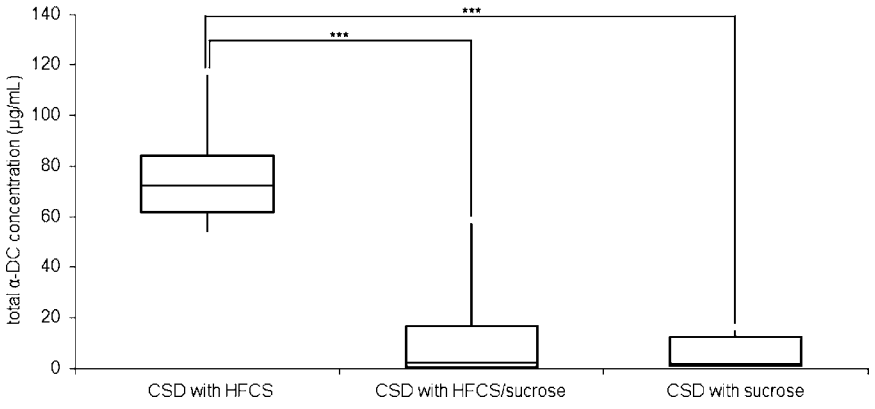


Figure 4. Box plot with whiskers from minimum to maximum of total α-dicarbonyl concentration in different groups of soft drinks clustered to the sort of sweetener. *** indicates highly significant differences with $p < 0.001$.

required that apply defined industrial processing to CSD raw material of defined composition to differentiate between carry-over of α-DCs from sweetener raw material and de novo formation from glucose and fructose during processing and storage of the CSD.

The UHPLC-DAD method established before to quantify seven different α-DCs (glucosone, 1-DG, 3-DG, 3-DGal, (*E*)-3,4-DGE, (*Z*)-3,4-DGE, and MGO) in HFCS[3] could not be directly applied to the analysis of CSDs because of interfering matrix components, particularly caffeine. Therefore, α-DCs had to be purified from the sample matrix by SPE prior to UHPLC analysis. The application of reversed phase SPE led to an excellent removal of interferents, simultaneously yielding α-DC recovery rates between 85.6 and 103.1% and a high reproducibility with RSD of six independent measurements of a CSD sample of <5%. Thus, the introduction of SPE into the UHPLC-DAD-MS/MS method allowed reliable quantification of six α-DCs in commercial CSD samples.

The most abundant α-DC proved to be 3-DG, followed by glucosone, 3-DGal, 1-DG, MGO, and 3,4-DGE. The concentrations of 3-DG and MGO were in a similar range as those reported previously.[4,5] Thornalley and Rabbani reported

somewhat lower MGO concentrations compared to Lo et al. However, only three batches of one brand have been analyzed in the former study for each HFCS-containing and sucrose-containing CSD. The present market overview revealed relatively high interbrand variations (<0.012−0.63 μg/mL MGO in HFCS-containing samples and <0.012−0.21 μg/mL MGO in sucrose-containing samples) so that the discrepancy of both studies could be caused by the selection of the CSD products.

In the present study, GO had to be excluded from quantification because of significant de novo formation from the highly concentrated glucose/fructose matrix during derivatization so that the result of GO quantification cannot be compared to previous studies.[4,5]

Very recently, 3-DG, 3-DGal, and MGO were analyzed in a range of food products.[29] In the soft drink samples, no MGO was detected, whereas 3-DG and 3-DGal were in a similar concentration range as determined in the present study for soft drinks, sweetened with HFCS/sucrose or sucrose alone.

Glucosone, 1-DG, and 3,4-DGE have been quantified for the first time in CSD, because they contribute considerably to the overall α-DC load in the samples. Mittelmaier et al. have shown that the glycating activity of single α-DCs differs to a great degree.[13] Similar differences may most likely be expected for the biological activity of α-DCs, indicating that even lower concentrated α-DCs may be relevant for product safety and quality due to their higher reactivity.[14,30] Therefore, further studies are required to determine the relevance of specific α-DCs for food safety and quality taking into account the concentration and relative activity of each compound.

The α-DC concentration was determined in different types of CSDs sweetened with either HFCS, a mixture of HFCS and sucrose, or sucrose alone. As expected, the highest α-DC concentrations were detected in HFCS-sweetened CSD samples. The other two product groups contained significantly lower amounts. HFCS consists of a mixture of the two reducing sugars fructose and glucose, which are readily degraded during the processing of HFCS,[3] but probably also during the preparation or storage of the soft drink. Formation of α-DCs from glucose and fructose, however, is dependent on the pH value. It was shown, for example, that the formation of 3-DG strongly decreases with decreasing pH in a range between pH 5.0 and 2.5.[31] Therefore, α-DC generation in acidic CSDs may be relatively low. The nonreducing disaccharide sucrose is not directly degraded, resulting in considerably lower concentrations of α-DCs. However, at the acidic pH value of CSDs, sucrose can be hydrolyzed to some extent, releasing glucose and fructose,[2] which can be partly converted to α-DCs.

The findings reported here demonstrate that almost all tested CSDs contain considerable amounts of glucosone, 1-DG, 3-DG, 3-DGal, 3,4-DGE, and MGO. The total amount of α-DCs is dependent on the applied sweetener. CSDs are consumed worldwide and play an important role in nutrition. Therefore, sugar-sweetened CSDs have to be considered as an important extrinsic source of α-DCs. Further research, however, is warranted to assess physiological consequences of the nutritive uptake of α-DCs.

AUTHOR INFORMATION

Corresponding Author

*Phone: +49-9131-8524102. Fax: +49-9131-8522587. E-mail: monika.pischetsrieder@fau.de.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

CSD, carbonated soft drinks; HFCS, high-fructose corn syrup; α-DC, alpha-dicarbonyl compound; UHPLC-DAD-MS/MS, ultrahigh-performance liquid chromatography with hyphenated diode array detector−tandem mass spectrometry; 3-DG, 3-deoxyglucosone; 3-DGal, 3-deoxygalactosone; 1-DG, 1-deoxyglucosone; 3,4-DGE, 3,4-dideoxyglucosone-3-ene; RSD, relative standard deviation; GO, glyoxal; MGO, methylglyoxal; AGEs, advanced glycation end-products; SPE, solid phase extraction; OPD, *o*-phenylenediamine; DTPA, diethylenetriaminepentaacetic acid; ESI, electrospray ionization; LOD, limit of detection; LOQ, limit of quantification

REFERENCES

(1) Economic Research Services (ERS) of U.S. Department of Agriculture (USDA). Food availabitily (per capita) data system; http://www.ers.usda.gov/data/foodconsumption (accessed Sept 16, 2011).,

(2) White, J. S. Straight talk about high-fructose corn syrup: what it is and what it ain't. *Am. J. Clin. Nutr.* **2008**, *88*, 1716S−1721S.

(3) Gensberger, S.; Mittelmaier, S.; Glomb, M. A.; Pischetsrieder, M. Identification and quantification of six major α-dicarbonyl process contaminants in high-fructose corn syrup. *Anal. Bioanal. Chem.* **2012**, *403*, 2923−2931.

(4) Lo, C.-Y.; Li, S.; Wang, Y.; Tan, D.; Pan, M.-H.; Sang, S.; Ho, C.-T. Reactive dicarbonyl compounds and 5-hydroxymethylfurfural in carbonated beverages containing high fructose corn syrup. *Food Chem.* **2008**, *107*, 1099−1105.

(5) Thornalley, P. J.; Rabbani, N. Dicarbonyls in cola drinks sweetened with sucrose or high fructose corn syrup. In *The Maillard Reaction: Interface between Aging, Nutrition and Metabolism*; Thomas, M., Forbes, J., Eds.; Royal Society of Chemistry: Cambridge, UK, 2010; pp 158−163.

(6) Ahmed, N.; Dobler, D.; Dean, M.; Thornalley, P. J. Peptide mapping identifies hotspot site of modification in human serum albumin by methylglyoxal involved in ligand binding and esterase activity. *J. Biol. Chem.* **2005**, *280*, 5724−5732.

(7) Niwa, T. 3-Deoxyglucosone: metabolism, analysis, biological activity, and clinical implication. *J. Chromatogr., B: Biomed. Sci. Appl.* **1999**, *731*, 23−36.

(8) Frischmann, M.; Bidmon, C.; Angerer, J.; Pischetsrieder, M. Identification of DNA adducts of methylglyoxal. *Chem. Res. Toxicol.* **2005**, *18*, 1586−1592.

(9) More, S. S.; Raza, A.; Vince, R. The butter flavorant, diacetyl, forms a covalent adduct with 2-deoxyguanosine, uncoils DNA, and leads to cell death. *J. Agric. Food Chem.* **2012**, *60*, 3311−3317.

(10) Breyer, V.; Frischmann, M.; Bidmon, C.; Schemm, A.; Schiebel, K.; Pischetsrieder, M. Analysis and biological relevance of advanced glycation end-products of DNA in eukaryotic cells. *FEBS J.* **2008**, *275*, 914−925.

(11) Shapiro, R.; Sodum, R. S.; Everett, D. W.; Kundu, S. K. Reactions of nucleosides with glyoxal and acrolein. *IARC Sci. Publ.* **1986**, 165−173.

(12) Tauer, A.; Knerr, T.; Niwa, T.; Schaub, T. P.; Lage, C.; Passlick-Deetjen, J.; Pischetsrieder, M. In vitro formation of Nε-(carboxymethyl)lysine and imidazolones under conditions similar to continuous ambulatory peritoneal dialysis. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1408−1414.

(13) Mittelmaier, S.; Pischetsrieder, M. Multistep ultrahigh performance liquid chromatography/tandem mass spectrometry analysis for untargeted quantification of glycating activity and identification of most relevant glycation products. *Anal. Chem.* **2011**, *83*, 9660−9668.

(14) Linden, T.; Cohen, A.; Deppisch, R.; Kjellstrand, P.; Wieslander, A. 3,4-Dideoxyglucosone-3-ene (3,4-DGE): a cytotoxic glucose

degradation product in fluids for peritoneal dialysis. *Kidney Int.* **2002**, *62*, 697−703.
(15) Leung, J. C.; Chan, L. Y.; Li, F. F.; Tang, S. C.; Chan, K. W.; Chan, T. M.; Lam, M. F.; Wieslander, A.; Lai, K. N. Glucose degradation products downregulate ZO-1 expression in human peritoneal mesothelial cells: the role of VEGF. *Nephrol. Dial. Transplant.* **2005**, *20*, 1336−1349.
(16) Mittelmaier, S.; Niwa, T.; Pischetsrieder, M. Chemical and physiological relevance of glucose degradation products in peritoneal dialysis. *J. Ren. Nutr.* **2012**, *22*, 181−185.
(17) Glomb, M. A.; Pfahler, C. Synthesis of 1-deoxy-D-erythro-hexo-2,3-diulose, a major hexose Maillard intermediate. *Carbohydr. Res.* **2000**, *329*, 515−523.
(18) Mittelmaier, S.; Funfrocken, M.; Fenn, D.; Fichert, T.; Pischetsrieder, M. Identification and quantification of the glucose degradation product glucosone in peritoneal dialysis fluids by HPLC/DAD/MSMS. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2010**, *878*, 877−882.
(19) Mittelmaier, S.; Funfrocken, M.; Fenn, D.; Pischetsrieder, M. 3-Deoxygalactosone, a new glucose degradation product in peritoneal dialysis fluids: identification, quantification by HPLC/DAD/MSMS and its pathway of formation. *Anal. Bioanal. Chem.* **2011**, *399*, 1689−1697.
(20) Bruggemann, L.; Quapp, W.; Wennrich, R. Test for non-linearity concerning linear calibrated chemical measurements. *Accredit. Qual. Assur.* **2006**, *11*, 625−631.
(21) de Revel, G.; Pripis-Nicolau, L.; Barbe, J.-C.; Bertrand, A. The detection of α-dicarbonyl compounds in wine by formation of quinoxaline derivatives. *J. Sci. Food Agric.* **2000**, *80*, 102−108.
(22) Glomb, M. A.; Tschirnich, R. Detection of α-dicarbonyl compounds in Maillard reaction systems and in vivo. *J. Agric. Food Chem.* **2001**, *49*, 5543−5550.
(23) Hollnagel, A.; Kroh, L. W. Formation of α-dicarbonyl fragments from mono- and disaccharides under caramelization and Maillard reaction conditions. *Z. Lebensm. Unters. Forsch. A* **1998**, *207*, 50−54.
(24) Mittelmaier, S.; Funfrocken, M.; Fenn, D.; Berlich, R.; Pischetsrieder, M. Quantification of the six major α-dicarbonyl contaminants in peritoneal dialysis fluids by UHPLC/DAD/MSMS. *Anal. Bioanal. Chem.* **2011**, *401*, 1183−1193.
(25) Marceau, E.; Yaylayan, V. A. Profiling of α-dicarbonyl content of commercial honeys from different botanical origins: identification of 3,4-dideoxyglucoson-3-ene (3,4-DGE) and related compounds. *J. Agric. Food Chem.* **2009**, *57*, 10837−10844.
(26) Bravo, A.; Herrera, J. C.; Scherer, E.; Ju-Nam, Y.; Rubsam, H.; Madrid, J.; Zufall, C.; Rangel-Aldao, R. Formation of α-dicarbonyl compounds in beer during storage of Pilsner. *J. Agric. Food Chem.* **2008**, *56*, 4134−4144.
(27) Daglia, M.; Papetti, A.; Aceti, C.; Sordelli, B.; Spini, V.; Gazzani, G. Isolation and determination of α-dicarbonyl compounds by RP-HPLC-DAD in green and roasted coffee. *J. Agric. Food Chem.* **2007**, *55*, 8877−8882.
(28) Hellwig, M.; Degen, J.; Henle, T. 3-Deoxygalactosone, a "new" 1,2-dicarbonyl compound in milk products. *J. Agric. Food Chem.* **2010**, *58*, 10752−10760.
(29) Degen, J.; Hellwig, M.; Henle, T. 1,2-Dicarbonyl compounds in commonly consumed foods. *J. Agric. Food Chem.* **2012**, *60*, 7071−7079.
(30) Witowski, J.; Korybalska, K.; Wisniewska, J.; Breborowicz, A.; Gahl, G. M.; Frei, U.; Passlick-Deetjen, J.; Jorres, A. Effect of glucose degradation products on human peritoneal mesothelial cell function. *J. Am. Soc. Nephrol.* **2000**, *11*, 729−739.
(31) Zimmeck, T.; Tauer, A.; Fuenfrocken, M.; Pischestrieder, M. How to reduce 3-deoxyglucosone and acetaldehyde in peritoneal dialysis fluids. *Perit. Dial. Int.* **2002**, *22*, 350−356.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on March 11, 2013, with errors to Table 5. The corrected version was reposted on March 18, 2013.