JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

1,2-Dicarbonyl Compounds in Commonly Consumed Foods

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Supporting Information

ABSTRACT: 1,2-Dicarbonyl compounds, formed from carbohydrates during thermal processing in the course of caramelization and Maillard reactions, are intensively discussed as precursors for advanced glycation endproducts in foods and in vivo. To obtain information about the uptake of individual compounds with commonly consumed foods, a comprehensive analysis of the content of 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), and methylglyoxal (MGO) together with 5-hydroxymethylfurfural (HMF) in 173 food items like bakery products, pasta, nonalcoholic and alcoholic beverages, sweet spreads, and condiments was performed. Following suitable cleanup procedures, 1,2-dicarbonyl compounds were quantitated after derivatization with *o*-phenylenediamine via RP-HPLC with UV detection. 3-DG proved to be the predominant 1,2-dicarbonyl compound with concentrations up to 410 mg/L in fruit juices, 2622 mg/L in balsamic vinegars, and 385 mg/kg in cookies, thus exceeding the corresponding concentrations of HMF. 3-DGal was found to be of relevance in many foods even in the absence of galactose. MGO was only of minor quantitative importance in all foods studied, except for manuka honey. Dietary intake was estimated to range between 20 and 160 mg/day for 3-DG and 5 and 20 mg/day for MGO, respectively.

KEYWORDS: *glucose degradation products, 1,2-dicarbonyl compounds, 3-deoxyglucosone, 3-deoxygalactosone, methylglyoxal, 5-hydroxymethylfurfural, quinoxaline, dietary intake*

INTRODUCTION

1,2-Dicarbonyl compounds are easily formed from sugars in caramelization reactions or during the Maillard reaction (also referred to as glycation).[1−3] They can be considered as intermediates in a complex reaction cascade where they are prone to react with mainly the N-termini and lysine and arginine side chains of proteins leading to "advanced glycation endproducts" (AGEs).[4] This wide range of products is discussed with either positive and negative consequences, both in food and in vivo.[5,6]

As so-called "glucose degradation products", 1,2-dicarbonyl compounds have been of major interest in glucose-containing peritoneal dialysis solutions because of possible adverse effects in vivo.[7] In recent years they have drawn more attention also in the field of food chemistry due to their role as precursors of color and aroma components and their ability to modify amino acid side chains of proteins.[4]

For the 1,2-dicarbonyl compounds formed from hexoses, structures with an "intact" C-6 backbone are known, such as 3-deoxyglucosone (3-DG) and glucosone, as well as breakdown products, e.g., following cleavage via retro-aldol reactions, like methylglyoxal (MGO) and glyoxal (GO).[8,9] Analogously, 3-deoxygalactosone (3-DGal) and galactosone originate from D-galactose.[10] From 1,4-glycosidically linked di- and oligosaccharides, predominately the C-5 compound 3-deoxypentosone (3-DPs) is formed.[11]

Due to their reactivity and their reversible binding to matrix components,[12] efficient analytical techniques are necessary for the quantitative determination of 1,2-dicarbonyl compounds in complex food matrices. A widely accepted technique to analyze 1,2-dicarbonyl compounds is based on their derivatization with *o*-phenylenediamine to the stable, UV-active quinoxalines (Figure 1). Separation of the quinoxalines formed is generally carried out via reversed-phase high performance liquid chromatography with UV detection (RP-HPLC−UV).[13]

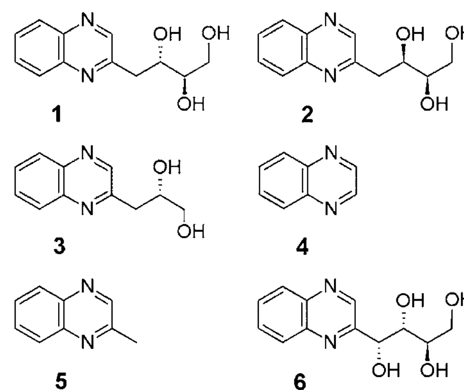

Figure 1. Chemical structures of corresponding quinoxalines of 1,2-dicarbonyl compounds obtained via derivatization with *o*-phenylenediamine: (**1**) 3-deoxyglucosone (3-DG), (**2**) 3-deoxygalactosone (3-DGal), (**3**) glucosone, (**4**) 3-deoxypentosone (3-DPs), (**5**) methylglyoxal (MGO), and (**6**) glyoxal (GO).

Detailed information about 1,2-dicarbonyl compounds in food is quite scarce and often limited to special food items like honey.[14,15] Particularly, quantitative data about short-chained MGO and GO are present in the literature. Small amounts ranging from 0.02 to 7.6 mg/kg or mg/L have been detected in fermented food like yogurt, alcoholic beverages, and soy

Received: March 28, 2012
Revised: June 21, 2012
Accepted: June 23, 2012
Published: June 23, 2012

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sauce.[16−18] For processed food like coffee[19,20] and baked cookies,[21] amounts between 23 mg/L and 210 mg/kg, respectively, were reported recently. Significant MGO contents of up to 740 mg/kg can be found in manuka honey from New Zealand.[22] In comparable ranges, 3-DG occurs in different kinds of honeys (from 80 mg to 1450 mg/kg), not only in manuka honeys.[14] Lo et al. reported high amounts of 3-DG in carbonated soft drinks containing high fructose corn syrup accompanied with high amounts of 5-(hydroxymethyl)-2-furfural (HMF), a stable end product of 3-DG.[23] Apart from that, only very little data about 3-DG in food is available. Recent studies, however, indicate that 3-DG is one of the predominant 1,2-dicarbonyl compounds in beer[18] and several other foods, especially lactose-hydrolyzed milk products.[10] It was therefore the aim of this study to investigate the occurrence of 1,2-dicarbonyl compounds (3-DG, 3-DGal, 3-DPs, MGO, and GO) in a great variety of commonly consumed foods such as beer, jams, spreads, juices, and bakery products. Based on this comprehensive quantitative data, the daily dietary exposure was estimated in order to enable discussions concerning nutritional consequences.

■ MATERIALS AND METHODS

Materials. Chemicals of the highest purity available were purchased from standard suppliers: DETAPAC (diethylenetriaminepentaacetic acid), disodium hydrogen phosphate (Merck, Darmstadt, Germany); *o*-phenylenediamine, methanol, 5-hydroxymethylfurfural, quinoxaline, 2-methylquinoxaline (SAF, Steinheim, Germany); sodium dihydrogen phosphate (Grüssing, Filsum, Germany). Quinoxalines of 3-deoxyglucosone, 3-deoxygalactosone, and 3-deoxypentosone were synthesized as previously published.[10] The water used for the preparation of buffers, solutions, and HPLC solvents was obtained using a Purelab plus purification system (USFilter, Ransbach-Baumbach, Germany).

Food samples. A total of 173 food items was purchased from different local supermarkets, among them 5 sweets, 15 soft drinks, 23 fruit beverages, 31 alcoholic beverages, 23 sweet spreads (jams, honey) and sweeteners, 39 condiments, and 28 bakery products. Freshly made products like 3 coffee drinks and cooked products like pasta and potatoes (6 items) were collected in a public cafeteria.

Preparation of Food Samples for Analysis. Liquid samples were analyzed without further preparation, except for carbonated drinks and beer, which were freed from gas by use of an ultrasonic bath. 15−20 g of semifluid and sugar-rich samples (jams and honey) were mechanically homogenized in water by Ultra-Turrax mixing, if necessary, and then filled up to 100 mL in volumetric flasks. Candy samples were dissolved to 15% (w/v) solutions in water. Bakery products were mechanically chopped and homogenized by a customary kitchen machine. Bread was homogenized as a whole slice (crust and crumb), or crust and crumb were separately chopped, with the crust defined as the outermost layer of a slice 15 mm thick. The prepared samples were stored at −20 °C until analysis.

For protein precipitation, 500 μL of the stock solutions was mixed with 1 mL of methanol and left at −20 °C for 1 h. If necessary, stock solutions were diluted with water prior to protein precipitation. The mixtures were centrifuged at 10000*g* for 15 min. For the analysis of 1,2-dicarbonyl compounds, 500 μL of the supernatant was then mixed with 150 μL of 0.5 M sodium phosphate buffer, pH 7.0 and 150 μL of a 0.2% (w/v) *o*-phenylenediamine solution containing 18.5 mM DETAPAC. The mixture was kept in the dark overnight and membrane filtered (0.45 μm) before chromatographic analysis.

In order to analyze 1,2-dicarbonyl compounds in solid food, an extraction step is required. 500 mg of homogenized bakery products was weighed into a 6 mL plastic tube, and 3 mL of water was added. The suspension was mixed vigorously by vortex. After 1 h of extraction at room temperature, a portion of 3 mL of methanol was added and the mixture was allowed to precipitate for 1 h at −20 °C. The mixtures were centrifuged at 5000*g* for 20 min, and 500 μL of the supernatant was then used for derivatization with *o*-phenylenediamine as described above.

HPLC−UV Analysis of Quinoxalines Formed from 1,2-Dicarbonyl Compounds and *o*-Phenylenediamine. HPLC analysis was performed using a high pressure gradient system from Amersham Pharmacia Biotech (Uppsala, Sweden), consisting of a pump P-900 with an online degasser (Knauer, Berlin, Germany), and a UV detector UV-900. Chromatographic separation of quinoxalines was carried out at room temperature on a stainless steel column filled with Prontosil 60 Phenyl material (250 mm × 4.6 mm, 5 μm, Knauer, Berlin, Germany) with an integrated guard column (5 mm × 4 mm, Phenyl material) and an online filter (3 μm) between sample loop and column. Gradient separation was performed with 0.075% acetic acid (solvent A) and a mixture of 80% methanol and 20% solvent A (solvent B) as the eluents using a flow rate of 0.7 mL/min. Samples were injected at 10% B, and then the proportion of B was changed linearly to 50% in 25 min; after a holding time of 5 min, the proportion of solvent B was elevated to 70% over 4 min, held for another 10 min, and then changed again to 10% B in 4 min. Subsequently, the column was equilibrated at 10% B for 10 min.[10]

External calibration was carried out with the corresponding quinoxalines of 3-DG, 3-DGal, 3-DPs, GO, and MGO as described previously.[10]

The limits of detection (LOD) and quantitation (LOQ) in flour and artificial honey matrices (containing 46.5% fructose, 34.5% glucose, 1.5% sucrose, and 17.5% water) were calculated as the concentrations of the analyte necessary to show a peak at a signal-to-noise ratio of 3 and 10, respectively.

HPLC−UV Analysis of 5-(Hydroxymethyl)-2-furfural (HMF). Analysis of HMF was carried out as previously published[10] for all food samples. Liquid samples were applied to methanolic precipitation without further preparation. Stock solutions were made from sugar-rich and semisolid samples (10−15%, w/v) as described above. As well sample preparation for solid products was implemented as set out above.

HPLC-DAD−ESI-MS Analysis of Quinoxalines Derived from 1,2-Dicarbonyl Compounds. Analysis was carried out according to the method published by Hellwig et al.[10]

Data Analysis. Data are given as concentration ranges measured on different similar samples from different companies and expressed as mg/L of 1,2-dicarbonyl compounds in liquid samples and mg/kg in semisolid and solid samples.

For the determination of the interday repeatability, the same samples of different food items (malt beer, dark beer, pear butter, balsamic vinegar, soy sauce, ketchup, rye bread, and rusk) were derivatized five times on different days with subsequent analysis. The recovery of 3-DG, 3-DGal, 3-DPs, GO, and MGO was calculated from the slope of the recovery function after spiking wheat flour and artificial honey with ascending concentrations (13−158 μM) of dicarbonyl compounds, following sample preparation and derivatization with *o*-phenylenediamine. For assaying the influence of the ratio of sample and solvent on the yield of extracted 1,2-dicarbonyl compounds from bakery products, ascending amounts (300, 400, 500, 600, and 700 mg) of sample (mixed-grain rye bread and rusk) were weighed into 6 mL plastic tubes and applied to sample workup as described above.

Statistical Treatment. Comparisons of means between food groups were examined using Student's *t* test. Correlation analysis was performed using SPSS. For correlation the Spearman's rank correlation coefficient (ρ) was calculated. P values less than or equal to 0.05 were considered significant (two-tailed). Histograms (see Supporting Information) were created using OriginPro 8.6.

■ RESULTS AND DISCUSSION

Analysis of 1,2-Dicarbonyl Compounds: Method Validity and Reliability. In a recent study, 1,2-dicarbonyl compounds were analyzed in a variety of milk products after derivatization with *o*-phenylenediamine to the corresponding quinoxalines using RP-HPLC with a phenyl column, which

Table 1. Performance Parameters for the RP-HPLC Method, Shown for Two Different Matrices

	semisolid, sugar-rich products					bakery products				
	LOD[a]		LOQ[a]			LOD[a]		LOQ[a]		
	μmol/kg	mg/kg[b]	μmol/kg	mg/kg[b]	recovery[c] (%)	μmol/kg	mg/kg[b]	μmol/kg	mg/kg[b]	recovery[d] (%)
3-DG	2.90	0.47	9.56	1.55	102.9 ± 0.7	5.01	0.81	16.25	2.68	96.7 ± 2.4
3-DGal	2.94	0.48	9.69	1.58	103.6 ± 1.1	4.93	0.80	16.26	2.64	97.5 ± 2.6
3-DPs	8.59	1.13	28.35	3.74	91.9 ± 2.6	15.48	2.04	51.07	6.75	105.2 ± 3.1
GO	4.74	0.28	15.65	0.91	102.8 ± 0.5	5.22	0.30	17.22	1.00	85.8 ± 4.2
MGO	3.26	0.23	10.76	0.78	102.5 ± 0.4	6.26	0.45	20.65	1.49	83.2 ± 1.8

[a]LODs and LOQs were calculated on the basis of signal-to-noise ratio. [b]Values are expressed for the 1,2-dicarbonyl compounds. [c]Recovery was determined by addition of various concentrations (13−158 μM, $n = 3$) of 1,2-dicarbonyl compounds to 10% solution of artificial honey in water under inclusion of all steps of analysis. Values were calculated from the slope of the recovery function and are given in percent ± SE. [d]Recovery was determined by addition of various concentrations (13−79 μM, $n = 3$) of 1,2-dicarbonyl compounds to 500 mg of wheat flour under inclusion of all steps of analysis. Values were calculated from the slope of the recovery function and are given in percent ± SE.

Table 2. Relative Interday Repeatability Values (Coefficients of Variation in %) for the Analysis of 1,2-Dicarbonyl Compounds in Different Food Items[a]

	malt beer	dark beer	pear butter	balsamic vinegar	soy sauce	ketchup	rye bread	rusk
3-DG	3.6	3.6	0.5	1.8	3.1	3.0	5.0	1.5
3-DGal	4.0	3.5	1.6	5.3	3.1	9.2	5.5	6.5
GO	nd	nd	9.7	nd	8.2	1.8	nd	5.9
MGO	tr	nd	tr	7.5	3.4	nd	nd	4.7

[a]Relative interday repeatability achieved by working up different food samples five times on five different days and measurement on five different days; nd, not detectable; tr, traces, means amounts between LOD and LOQ calculated in this study and in standard solution as published recently.[10]

enabled the separation of epimeric quinoxalines and, therefore, a reliable quantitation of the analytes.[10] The occurrence of 3-DG in juices and beer as the major 1,2-dicarbonyl compound along with small amounts of 3-DGal[10] has been a motivation to prove these results and extend our research to a variety of food, especially to sugar-rich samples, semisolid sweet spreads, and solid bakery products. Therefore, the method had to be transferred from liquid to semisolid and solid samples, particularly concerning sample preparation.

For analysis of semisolid, sugar-rich materials like honey, aqueous solutions (10−15%, w/v) were prepared by dissolving the sample in water according to Weigel et al.[14] For fibered samples like jam, a homogenization step by Ultra-Turrax mixing after dissolution is required. The same procedure is carried out for solid material like candies.

For reasons of consistent sample preparation, the addition of methanol is carried out for all samples, even though the major part of samples does not contain substantial amounts of protein.

Method performance parameters are given in Table 1. Calibration was performed, according to Hellwig et al.,[10] with the corresponding quinoxalines of the 1,2-dicarbonyl compounds 3-DG, 3-DGal, 3-DPs, GO, and MGO. All calibration curves showed linear response as described in our recently published paper[10] within a range from 0.2 to 49 mg/L. LODs and LOQs determined in flour matrix and artificial honey matrix were higher when compared to recently published data for standard solutions.[10] LOQs ranging for 1,2-dicarbonyl concentrations in flour from 1.00 to 6.75 mg/kg (equivalent to 16.25 to 51.07 μmol/kg) and in artificial honey from 0.78 to 3.74 mg/kg (equivalent to 9.56 to 28.53 μmol/kg) were determined.

Recovery experiments were carried out for two different matrices and procedures of sample preparation: for sugar-rich, semisolid matrices like jam and honey, and solid material like bakery products. As for both of the matrices no analyte-free material was available, the matrices were simulated. As a representative of sugar-rich matrices, artificial honey was used. Wheat flour was chosen as a matrix simulating bakery products.

After spiking the exemplary matrices, samples were allowed to stand 1 h at room temperature prior to sample workup. For both matrices, good recoveries for all analyzed 1,2-dicarbonyl compounds were achieved ranging from 83.2 to 105.2% for different concentrations comparable to recoveries published in the literature.[10,21] These results show that the examined 1,2-dicarbonyl compounds can be discharged from semisolid and solid sample material during the applied dissolving and extraction steps. The slightly lower recoveries for MGO and GO in flour might be due to possible reactions of the reactive dicarbonyl compounds with matrix components like protein during sample preparation. Moreover, the influence of the employed ratio of bakery product and extractant was investigated. The results for the corresponding 3-DG and 3-DGal levels show a coefficient of variation of 5% or below. It is therefore concluded that the applied ratio of amount of bakery product and extractant does not affect the yield of extracted 1,2-dicarbonyl compounds in the tested ranges. The relative interday repeatability was assessed for all 1,2-dicarbonyl compounds by working up different food samples five times on five days and ranged between 0.5 and 9.7% irrespective of the determined concentrations (Table 2), confirming the accuracy of the presented amounts.

Based on the good performance of the validation parameters, the sample preparation for semisolid and solid food is considered to be suitable for accurate quantitation of 1,2-dicarbonyl compounds. Other parameters such as completeness of derivatization, generation of 1,2-dicarbonyl compounds during derivatization, and chromatographic separation have already been assessed in previous works.[10,14]

In order to avoid false positive results, all food samples were additionally measured without *o*-phenylenediamine derivatization in order to recognize possible coeluting substances

originating from the matrix. UV chromatograms of solutions of *o*-phenylenediamine at wavelengths 312 and 280 nm were recorded to rule out possible interfering compounds present in the derivatizing agent. Identification and peak assignment of the quinoxalines were verified with HPLC-DAD−ESI-MS on selected food samples. In Supplement 1 in the Supporting Information, HPLC-DAD−ESI-MS data (UV chromatograms at 312 nm as well as corresponding UV and mass spectra) of two representative food items (balsamic vinegar and soy sauce) are shown. During quantification of dicarbonyl compounds, chromatograms for all samples were recorded at 312 and 280 nm simultaneously and the ratio between peak areas obtained at the two wavelengths was calculated as indicator for peak purity and identity.

Analysis of 1,2-Dicarbonyl Compounds in Different Food Products. In this study, a broad range of daily consumed foods was analyzed including liquid, semisolid, and solid samples. Most samples chosen for the study were liquid and could easily be introduced into the analysis protocol; solid and semisolid samples were dispersed in water or homogenized by Ultra-Turrax mixing. Samples like mustard could not be measured using UV detection because of coeluting matrix constituents, which were identified from the chromatograms of nonderivatized samples. MS detection should be performed on such samples. In Figure 2, exemplary chromatograms of a cherry jam, a honeydew honey, and a balsamic vinegar sample are displayed. For peak assignment, a chromatogram of a standard mixture of the five analyzed 1,2-dicarbonyl compounds is shown. 3-DG is the dominating 1,2-dicarbonyl compound in these samples, with concentrations up to 2622 mg/L in balsamic vinegar. The highest level recorded for 3-DGal in this study was 162 mg/kg in balsamic vinegar and 463 mg/kg for MGO in manuka honey, corresponding with the literature.[22]

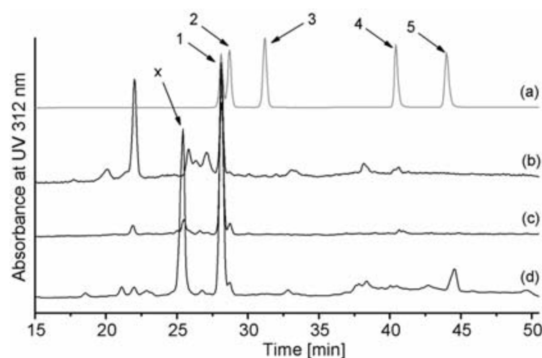

Figure 2. Chromatograms of (a) a standard quinoxaline mixture, (b) cherry jam, (c) honeydew honey, and (d) balsamic vinegar after sample preparation and derivatization with *o*-phenylenediamine acquired by RP-HPLC with UV detection (312 nm). Arrows indicate the peaks of the quinoxalines of (**1**) 3-deoxyglucosone, (**2**) 3-deoxygalactosone, (**3**) 3-deoxypentosone, (**4**) glyoxal, (**5**) methylglyoxal, and (x) unknown peak in samples (b), (c), and (d).

In Table 3, results for 3-DG, 3-DGal, MGO, and HMF for different food categories are displayed as ranges of values each with median level. Additionally, histograms of the 3-DG content of different food groups (where number of samples was $n \geq 8$) are displayed in Supplement 2 in the Supporting Information. When comparing the occurrence of 3-DG in nonalcoholic beverages, significantly higher amounts can be observed in fruit juices than in soft drinks, reaching up to 410 mg/L in prune juice accompanied by high values of HMF, though the sugar content is equal (about 100 g/L). This finding is in accordance with literature reports,[24,25] revealing high amounts of HMF in dried prunes (237−467 mg/kg) and juice made therefrom (1577 mg/L). The concentrations of 3-DG in fruit juices did not show any dependence on the fruit content, and there were no significant differences between direct juices and juices from concentrate; 20 of the 23 fruit juices have a 3-DG content equal to or less than 60 mg/L (see Supplement 2 in the Supporting Information). One reason for the difference of 3-DG contents between soft drinks and juices is the presence of monosaccharides in juices, like glucose and fructose, originating from the starting fruit material. On the contrary, in soft drinks, sucrose is normally used for sweetening purposes (on the German market); this sugar is less susceptible to degradation than other mono- and disaccharides due to its full acetal structure. Highest amounts of 3-DG in soft drinks are detected when glucose−fructose syrup is used as an additional sweetener (3.4−28 mg/L), as has also been reported in the literature;[23] but on the contrary, MGO was not detectable in our studies in glucose−fructose syrup containing soft drinks. 3-DGal in fruit juices might originate from traces of galactose present in fruits. High amounts present in prune juice (60 mg/L) can be explained by the epimerization of 3-DG via 3,4-dideoxyglucoson-3-ene (3,4-DGE) as implied in the literature.[10,18,26] HMF was detectable in 15 of 22 juices, with concentrations up to 714 mg/L, but the median level (1.0 mg/L) is much lower than the 3-DG concentrations. This is in line with our recent findings concerning HMF in honey. During heating or storage, 3-DG as a kinetically stable intermediate can accumulate, due to delayed generation of thermodynamically stable HMF.[10,14] The observed differences between the values within a group can indicate a carryover effect of 1,2-dicarbonyl compounds from added ingredients, which themselves contain these compounds.[23]

Contrary to the literature,[19,20] no 1,2-dicarbonyl compounds, neither 3-DG, nor MGO, nor GO, could be detected in different kinds of coffee drinks (filter coffee, cafè crema, and espresso) in this study. Analysis in coffee samples can be hindered by a complex sample matrix. Daglia et al.[20] employed solid phase extraction on coffee samples to eliminate the influence of matrix components on the detection. In this study, only a dilution step was applied to reduce the matrix influence, which could have led to 1,2-dicarbonyl compounds below LOQ or LOD. Further studies using liquid chromatography with more sensitive MS/MS detection should be performed on these samples. Published contents of MGO in different coffee drinks were between 4 and 47 mg/L,[19,27] but no values for 3-DG were reported in the literature.

Other than soft drinks and fruit juices, alcoholic beverages are characterized by a fermentation step in their manufacturing process, which should influence the concentrations of 1,2-dicarbonyl compounds. As can be seen from Table 3, beers, irrespective of their color intensity, did not differ strongly in their 3-DG concentrations (see Supplement 2 in the Supporting Information). 3-DGal was quantified in 9 of 10 beer samples in concentrations up to 16 mg/L. Interestingly, the 3-DG contents in beer are only 3−4 times higher than the 3-DGal contents, while in fruit juices, 50 times higher amounts of 3-DG compared to 3-DGal were found. In parallel, only small concentrations of HMF comparable with data published by Husøy et al.[24] were measured in beer. We believe that this is

Table 3. Amounts of 1,2-Dicarbonyl Compounds in Different Food Items

		3-DG[a]		3-DGal[a]		MGO[a]		HMF[a]	
food product	*n*	range	median	range	median	range	median	range	median
soft drinks	11	nd−28	1.6	nd−3.1	nd	nd	nd	nd−2.3	0.3
juices	23	nd−410	27	nd−60	1.3	nd−2.2	nd	nd−714	1.0
coffee	3	nd	nd	nd	nd	nd	nd	4.5−29	10
malt beer	4	19−136	30	4.8−33	11	tr.-1.0	0.7	3.0−25	5.5
beer	10	18−54	34	nd−16	11	nd−1.0	0.5	0.9−5.3	1.4
wine	21	2.2−95	7.0	nd−49	nd	nd−4.5	nd	nd−133	nd
vinegars	23	4.6−2622	341	1.1−162	14	1.7−53	8.9	0.6−3760	123
soy sauces	6	32−832	84	12−71	17	nd−12	8.1	nd−5.7	nd
liquid condiments/seasonings	10	nd−212	16	nd−22	nd	nd−3.9	nd	nd−122	4.9
jams, jellies, sweeteners	19	1.7−1061	165	nd−124	8.7	nd−13	3.6	nd−581	16
honey	4	271−1641	626	14−46	34	nd−463	1.0	nd−9.6	3.6
candies	5	141−1011	242	nd−36	6.9	nd−1.1	nd	nd−13	7.7
bread	12	13−619	45	nd−47	4.8	nd−28	3.0	1.9−163	5.9
alkali-treated pretzel	3	4.5−34	27	tr−6.4	6.2	2.5−16	14	0.9−3.1	2.6
cookies	13	8.5−385	129	tr−88	14	1.8−68	8.3	0.3−448	2.2
pasta (cooked)	3	nd−8.8	1.2	nd	nd	nd	nd	nd	nd
potatoes (cooked/fried)	3	nd−18	6.9	nd	nd	nd−tr	nd	nd	nd

[a]Data are given as ranges in mg/L or mg/kg, respectively; *n*, number of samples; nd, not detectable; tr, traces, means amounts between LOD and LOQ calculated in this study and in standard solution as published recently.[10]

strong evidence for the above-mentioned epimerization of 3-DG to 3-DGal, as hypothesized by Bravo et al.[18] and Hellwig et al.[10] The dehydration of intermediate 3,4-DGE should be hindered in aqueous systems like juices or beer, so that any thermal impact during food processing should rather induce 3-DGal rather than HMF formation. MGO was detected in 6 of 10 samples. This short-chain 1,2-dicarbonyl compound can originate from the fermentation process and has already been analyzed in similar concentrations in beer and wine by other groups.[16,17] While 3-DG was detected in all wine samples (15 of 21 samples showing 3-DG values equal to or less than 10 mg/L, see Supplement 2 in the Supporting Information), 3-DGal was found only in two samples of white wine (3.4 mg/L) and red wine (1.6 mg/L). The concentrations were higher when fortified wines (Sherry, Madeira) were analyzed, where 3-DG levels reached 67−95 mg/L and 3-DGal levels were between 4.7 and 49 mg/L. In these samples, also higher concentrations of HMF were measured, possibly because 3,4-DGE increasingly tends to react to HMF rather than to 3-DGal when it becomes more concentrated and water becomes more limited. Increased concentrations of HMF in fortified wines and liqueurs are known from the literature.[28] The relatively high concentration of MGO in fortified wines[17] could as well be corroborated in this study.

Vinegars, especially balsamic vinegars, and soy sauces show high 3-DG levels due to the manufacturing process, like cooking of must, ripening, and fermentation. Values ranging from 32 to 832 mg/L were determined in soy sauce. High 3-DG levels in soy sauce may also originate from caramelized sugar sources or other intensely heated carbohydrate sources, for instance caramel or molasses, which had been added as an ingredient. The group of vinegars covers a wide range of 3-DG levels from 5 to 2622 mg/L, but if the frequency distribution of the values is taken into account the majority of 3-DG levels (18 out of 23) are below 500 mg/L (see Supplement 2 in the Supporting Information). Low 3-DG values occur in wine vinegars and white balsamic vinegars (medians 33 mg/L and 49 mg/L), but high levels can be measured in balsamic vinegars (median 361 mg/L). 3-DG values vary considerably in balsamic vinegars in comparison to wine and white balsamic vinegars, which can be attributed directly to broader varieties in manufacturing process and added additives like caramel syrup. Balsamic vinegar was rich in HMF (median 293 mg/L) and showed high MGO contents (up to 53 mg/L). Taken together, balsamic vinegar is not only rich in HMF, but contains substantial amounts also of other sugar degradation products. For the 16 balsamic vinegars analyzed in this study, either traditionally manufactured or industrially produced, the summed concentration of sugar degradation products is between 221 and 6563 mg/L (median 729 mg/L). The concentration of sugar degradation products in these samples can exceed the concentration of, e.g., Amadori products—the most important representatives of the Maillard reaction—in heat-treated foods like condensed milk and bakery products.[29]

MGO levels of all types of vinegars (wine, white balsamic, balsamic) show similar ranges. This is assumed to indicate the different pathways of formation of long- and short-chained 1,2-dicarbonyl compounds and the relevance of fermentation processes for the formation of short-chained 1,2-dicarbonyls. MGO was also quantitated in all soy sauces and was present in the concentration range already published in the literature.[19] All the other condiments, like pepper sauce, ketchup, or oyster sauce, showed significantly lower 1,2-dicarbonyl compound contents (3-DG: median 16 mg/L), since they lack the relevant manufacturing processes like fermentation, ripening, or long, intense heating. As 1,2-dicarbonyl compounds are known precursors of aroma-active compounds,[18,30] we conclude that the 1,2-dicarbonyl content must have a direct impact on the aromatizing capabilities of condiments like soy sauce or vinegar, especially balsamic vinegar.

The dicarbonyl compound 3-deoxypentosone, resulting mainly from the degradation of 1,4-glycosidically linked di- and oligosaccharides,[11] has recently been detected in heated glucose−lysine solutions.[9] Analyzing 3-DPs was difficult in complex food samples because of coeluting matrix peaks (e.g., vanillin), which could be revealed by deviations in their UV absorbance pattern. In samples of soy sauce, however, 3-DPs could be identified using mass spectrometry (see Supplement 1

dx.doi.org/10.1021/jf301306g | J. Agric. Food Chem. 2012, 60, 7071−7079

in the Supporting Information) and quantified (see Figure 3), with values ranging from 10 to 40 mg/L. In the displayed

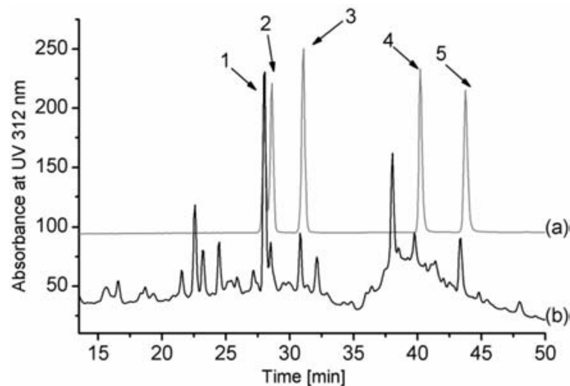

Figure 3. Chromatograms of (a) a standard quinoxaline mixture and (b) soy sauce after sample preparation and derivatization with *o*-phenylenediamine acquired by RP-HPLC with UV detection. For peak assignment compare Figure 2.

chromatogram of balsamic vinegar (Figure 2), a major unknown peak eluting at 25.1 min was detectable (in addition to the predominant peak of the 3-DG quinoxaline), which is not present in the underivatized sample. First investigations indicate that this peak could be a product formed from HMF and *o*-phenylenediamine during the derivatization reaction. Incubation of a standard solution of HMF with *o*-phenylenediamine leads to a peak showing the same retention time as the peak in the balsamic vinegar as well as a UV absorbance at 312 nm higher than at 280 nm which is characteristic for quinoxalines and substances with quinoxaline-like structures. ESI-MS analysis of this peak revealed a mass-to-charge ration m/z of $[M + H]^+ = 215.1$. Further investigations should be performed to elucidate the structure of the eluting substance.

1,2-Dicarbonyl compounds were then determined in different types of sweet spreads, honeys, and sweeteners like maple syrup. These 23 samples represent sugar-rich samples (c = 600−800 g/kg) with limited water activity. 3-DG and 3-DGal were present in all 10 samples of jams and jellies in concentrations ranging from 72 to 301 mg/kg and 1.9 to 42 mg/kg, respectively. Honeydew honey showed a high level of 3-DG (1641 mg/kg), which is in agreement with published results.[14] Moreover, the presence of 3-DGal in honey (14−46 mg/kg) could unequivocally be highlighted in this work (Figure 2) for the first time.

Plum butter and pear butter are spreads which are thickened by prolonged cooking. Sugar beet syrup is made of molasses from industrial sugar production. Such products are therefore exposed to much higher thermal impact than jams, which is directly reflected by the concentrations of 3-DG, 3-DGal, and HMF. For example, in sugar beet syrup, high contents of 3-DG (1025 mg/kg), 3-DGal (124 mg/kg), and HMF (239 mg/kg) were measured in this study. Among sweet spreads and sweeteners, lowest 3-DG levels were detected in two maple syrups (1.7−4.7 mg/kg). This observation can be directly linked to the fact that sucrose is the major sugar in maple syrup and only small amounts of monosaccharides are present. For frequency distribution of the 3-DG contents see Supplement 2 in the Supporting Information. MGO levels detected in jams were low, as well as MGO levels in honey, except for manuka honey. In this manuka honey sample, the highest MGO content (463 mg/kg) was measured in this study in accordance with the values published recently by Mavric et al.[22] While HMF contents ranged between not detectable and 32 mg/kg for jams (median 16 mg/kg), approximately 20 times more of the thermodynamically stable HMF was determined in intensely thermally treated plum and pear butters and sugar beet syrup (239−581 mg/kg). On the contrary, only very little HMF was detected in honey (< 9.6 mg/kg), which is not heated during processing. The HMF values measured for jams in this study are in line with recently published amounts.[31]

Analysis of 1,2-Dicarbonyl Compounds in Bakery Products. Sample preparation for bakery products could be properly integrated in the protocol of analysis of 1,2-dicarbonyl compounds in food. Recovery experiments indicate a complete and reproducible extraction of the analytes from a solid, flour-based matrix (see Table 1). Then, 1,2-dicarbonyl compounds were analyzed in rolls made of different flour types (wheat, rye, whole grain with malt and spelt), in mixed-grain bread, pumpernickel, rusk, and 13 different kinds of cookies. 3-DG was present in rolls and bread in only minor concentrations, independent of the flour type (13−67 mg/kg). When 3-DG was analyzed not in a homogeneous mixture of crust and crumb, but separately, then significantly higher values were detected for 3-DG in the crust than in the crumb (22 mg/kg versus 3.3 mg/kg; $p < 0.05$) (Figure 4). This is due to more

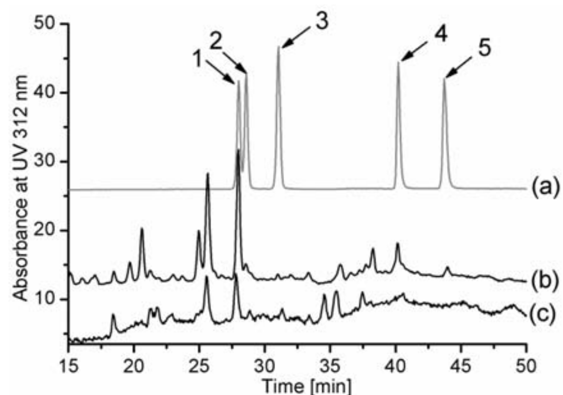

Figure 4. Chromatograms of (a) a standard quinoxaline mixture, (b) rye bread crust (15 mm thick), and (c) rye bread crumb after sample preparation and derivatization with *o*-phenylenediamine acquired by Phenyl-RP-HPLC with UV detection. For peak assignment see Figure 2.

intensive heat impact during the baking process in the outermost layer of the bread. 3-DGal was detected only in one crumb sample, but in all crust samples; the same applies to MGO. Maximum levels of 3-DG were detected in rusk (up to 619 mg/kg) accompanied by elevated levels of MGO (28 mg/kg). These samples contain glucose or invert sugar syrup, and intense heat treatment is part of their manufacturing process. Besides, we expected 3-DPs as a major 1,2-dicarbonyl compound in bakery products due to its role as a specific degradation product of di- and oligosaccharides having 1−4-glycosidic linkage,[11] but in the analyzed samples, 3-DPs was only detectable in alkali-treated pretzels (up to 2.3 mg/kg). This could be associated with the alkaline pH-value in these samples, as fragmentation of carbohydrates is favored under alkaline conditions.[30] Among the 13 cookies analyzed in this study were different types like shortbread, cream-filled biscuits, and cookies made of oat flour. Slightly higher 3-DG levels were

present in cookies than in bread (not significant, $p > 0.05$, see frequency distribution in Supplement 2 in the Supporting Information). 3-DGal and MGO were detectable in every sample. Median levels detected for MGO were slightly lower than recently reported in the literature.[21] The difference in 1,2-dicarbonyl amounts between bread and cookies mainly results from added ingredients. Cookies contain high amounts of sweeteners (210−470 g/kg), among which sucrose is the most important, but increasingly glucose syrup or invert sugar syrup are used as well. However, normally no sugar is added to bread. In a cookie containing caramel syrup, maximum 3-DG level was detected; probably 3-DG not only was generated during manufacturing but is a result of carryover from the caramel syrup. Though cookies contain high amounts of sugars and are exposed to intensive heat treatment during baking, smaller amounts of 3-DG were detected in comparison to candies and jams. This can be due to the possibility of Maillard reactions in bakery products: Not only does the presence of amines promote sugar degradation, but the amines can also be modified by dicarbonyl compounds. 3-DG and, probably, 3-DGal as well react with lysine side chains of proteins to form the advanced glycation end product pyrraline, which is comparatively stable and can serve as an integral parameter for 3-DG formed during heating processes. The pyrraline content in bread crust can yield values up to 3680 mg/kg protein,[32] which is equivalent to 250−300 mg/kg crust, assuming a protein content of 7−8%. Thus, at least 160−190 mg/kg 3-DG must have been formed and reacted with lysine residues. Since 3-DG is able to react with arginine as well, the "true" amount of 3-DG formed during baking will still be higher. The HMF contents of bakery products measured in this study correspond with data published in the literature reporting values for bread ranging between 3.4 and 69 mg/kg[33] and for cookies from 0.5 to 75 mg/kg.[34]

In staples like cooked pasta or potatoes, only 3-DG was detectable in minor amounts. On the one hand this could be linked to the formation of protein-bound lysine and arginine adducts,[32] and on the other hand free dicarbonyl compounds could be extracted into the water during the boiling process. Probably higher concentrations of dicarbonyl compounds should be detected when analyzing dried pasta.

In summary, the data presented here show that, among these compounds, 3-DG is of paramount importance in food systems and exceeds concentrations of HMF not only in honey.[14] Moreover, 3-DGal has been quantitated in foods in concentrations also ranging above those of MGO as one of the short-chain 1,2-dicarbonyl compounds which have mainly been analyzed in the past.[16,17,19,20,22] An important source of 3-DGal must obviously be galactose, e.g., in lactose-hydrolyzed milk products.[10] On the other hand, we agree with Bravo et al.,[18] who stated that 3-DGal can be formed by epimerization from 3-DG. The interconversion of these kinetically stable compounds is favored as long as they are diluted and no strong heat treatment is applied. When foods are heated and/or water becomes limited, the intermediate 3,4-DGE dehydrates and forms the thermodynamically stable end product HMF.

Altogether some conclusions can be drawn about the conditions in food samples which exert major influence on the occurrence of 1,2-dicarbonyl compounds in these, in particular on the 3-DG level. (I) The most important factor is the type of sugar (monosaccharide versus disaccharide and reducing versus nonreducing sugar) present in the food sample. (II) The extent of heat impact during manufacturing accompanied by a reduction of the water content plays a pivotal role. (III) Processes like ripening, fermentation, and storage promote the formation of 1,2-dicarbonyl compounds. (IV) 3-DG can accumulate in samples where no reactants like amino acid side chains of proteins are present.

Furthermore, varying contents of 1,2-dicarbonyl compounds in the same group of food items (e.g., balsamic vinegar, soft drinks) can on the one hand be attributed to differences in the manufacturing process and on the other hand linked to a carryover effect of added ingredients which themselves contain 1,2-dicarbonyl compounds (e.g., glucose−fructose syrup, molasses, or caramel).[23] Low levels of MGO can be due to the reactivity of that molecule and delayed formation in the degradation of sugars.

Daily Intake of 1,2-Dicarbonyl Compounds. Based on these extended quantitative data about 1,2-dicarbonyl compounds in a variety of food, it was possible to make a rough estimate of the daily intake of 1,2-dicarbonyl compounds from common diets. In Table 4, contents of 1,2-dicarbonyl compounds and HMF in common serving sizes of different foodstuffs are presented. From these values it is particularly obvious that the ingested amounts of 3-DG exceed those of the other 1,2-dicarbonyl compounds and HMF about 10-fold. MGO is taken up in only very small amounts. As the content of 1,2-dicarbonyl compounds and HMF per serving size is calculated on the basis of median levels, intake per serving size can vary strongly depending on the sample, as the example of balsamic vinegar shows: The lowest 3-DG level of balsamic vinegar measured in this study would lead to an intake of 7 mg and the highest level to an intake of 79 mg per serving size. As can be seen from the values of Tables 3 and 4, 3-DG is mainly ingested via sugar-rich food like honey and jam, as well as via fermented food like balsamic vinegar. These food products are not consumed to a large extent, but they preponderate because of their high amounts of 3-DG. Another important source of ingested 3-DG are beverages like fruit juices or beer, though they are not containing high levels of 3-DG but they are consumed in larger quantities. Staples are consumed in large quantities, but only contain medium amounts of 3-DG. Therefore, items like bread lead only to a little intake of the compound. Moreover, other staples like pasta or potatoes do

Table 4. Contents of 1,2-Dicarbonyl Compounds in Different Foods, Calculated Per Serving Size

food product	serving size	3-DG[a]	3-DGal[a]	MGO[a]	HMF[a]
softdrink	300 mL	0.5	nd	nd	0.1
fruit juice	300 mL	8.1	0.4	nd	0.3
malt beer	500 mL	15	5.7	0.3	2.8
beer	500 mL	17	5.6	0.2	0.7
wine	200 mL	1.4	nd	nd	nd
jam	20 g	3.3	0.2	0.1	0.3
honey	20 g	13	0.7	nd	0.1
candies	40 g	9.7	0.3	nd	0.3
balsamic vinegar	30 mL	10.2	0.4	0.3	3.7
soy sauce	30 mL	2.5	0.5	0.2	nd
bread	120 g	5.4	0.6	0.4	0.7
cookies	50 g	6.5	0.7	0.4	0.1
pasta (cooked)	250 g	0.3	nd	nd	nd
potatoes (cooked)	250 g	1.7	nd	nd	nd

[a]Data are given in mg, based on the median level of the values; nd, not detectable.

not contain substantial levels of 3-DG. In the literature, espresso or coffee is considered as a principal source for short-chain dicarbonyl compounds like MGO and GO.[19,20,27] In our study, however, we were not able to corroborate these findings due to analytical limitations. Therefore, a final statement on the contribution to the daily intake of MGO and GO cannot be made.

These elementary considerations concerning the dietary intake of 3-DG and MGO were based on a rough estimation of the 1,2-dicarbonyl compounds in hypothetical diets. For the minimum intake of 3-DG we hypothesized a diet mainly based on fresh fruits, vegetables, and milk products; for the maximum 3-DG intake a diet rich in sugar-rich products like sugar beet syrup together with the consumption of fruit juices and beer was assumed. Based on this, for 3-DG a total dietary intake between 20 and 160 mg/day (0.1−1 mmol/day) can be estimated, with an average intake of about 50 mg/day. The daily intake of MGO was rated between 5 and 20 mg per day (0.1−0.3 mmol/day). To the best of our knowledge there is no information about the bioavailability and the intestinal absorption of alimentary 1,2-dicarbonyl compounds available. For this reason, concluding statements about possible nutritional consequences resulting from ingested 1,2-dicarbonyl compounds are not possible. However, when comparing the ingested alimentary levels of 3-DG and MGO with corresponding levels of these compounds found in vivo, it becomes evident that the dietary intake of these compounds far exceeds the total load of the human body, present as free dicarbonyl compounds in blood plasma, For healthy individuals, plasma concentrations between 0.04 and 0.65 μM for 3-DG and MGO, respectively, were reported.[35−38] When compared with the high concentrations in several food items and the above-mentioned dietary intake, these low physiological concentrations may indicate that the alimentary 1,2-dicarbonyl compounds are not absorbed in the gastrointestinal tract and/or that the human body efficiently metabolizes these compounds via catabolic systems. As the intake of 1,2-dicarbonyl compounds seems to be inevitable, we are convinced that catabolic systems for these substances described in vivo[39−41] will also be effective at detoxifying such compounds from food. Corresponding studies on the metabolic transit of dietary 1,2-dicarbonyl compounds are underway in our laboratory.

ASSOCIATED CONTENT

Supporting Information

Additional experimental details as discussed in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was supported by the European Social Fund and the Free State of Saxony (SAB-Nr. 80941653).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

3,4-DGE, 3,4-dideoxyglucoson-3-ene; 3-DG, 3-deoxyglucosone; 3-DGal, 3-deoxygalactosone; 3-DPs, 3-deoxypentosone; AGE, advanced glycation end product; DETAPAC, diethylenetriaminepentaacetic acid; ESI-MS, electrospray ionization coupled with mass spectrometry; GO, glyoxal; HMF, 5-hydroxymethylfurfural; HPLC, high pressure liquid chromatography; LOD, limit of detection; LOQ, limit of quantitation; MGO, methylglyoxal; nd, not detectable; RP, reversed phase; tr, trace amount

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