AGRICULTURAL AND FOOD CHEMISTRY

Reactivity of Thermally Treated α -Dicarbonyl Compounds

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ABSTRACT: The degradation reaction of thermally treated 3-deoxy-D-*erythro*-hexos-2-ulose and methylglyoxal, both key intermediates in Maillard chemistry, was investigated. Different analytical strategies were accomplished to cover the broad range of formed products and their different chemical behavior. These involved HPLC-DAD and accordingly LC/MS analysis of the quinoxaline derivates, GC/MS analysis of the acetylated quinoxalines, and GC-FID analysis of the decyl ester of acetic acid. As a main degradation product of 3-deoxy-D-*erythro*-hexos-2-ulose, 5-(hydroxymethyl)furfural could be identified. At alkaline pH values, 3-deoxy-D-*erythro*-hexos-2-ulose generated various acids but no colored products. In contrast, thermal treatment of methylglyoxal yielded high molecular weight, brownish products. A dimer of methylglyoxal, first precursor for aldol-based polymerization of methylglyoxal, could be clearly identified by GC/MS.

KEYWORDS: α -dicarbonyl compounds, 3-deoxy-D-erythro-hexos-2-ulose, methylglyoxal, Maillard reaction

INTRODUCTION

The term nonenzymatic browning comprises caramelization and the Maillard reaction as the most important reactions.^{1,2} The Maillard reaction is a complex sequence of various reactions, in which amino acids and reducing carbohydrates are transformed into new components by heating. Because almost every food contains carbohydrates, amino acids, and proteins, respectively, this reaction plays a central role for manufacturing, processing, and storage.³ α -Dicarbonyl compounds are key intermediates of the Maillard reaction. These are significant for flavor and color as well as for the stability of food.^{4,5} For example, the Amadori compound of D-glucose delivers 1deoxyhexodiulose via the Amadori–Heyns rearrangement and subsequent 2,3-enolization.^{3,6} This well-investigated compound is converted to various products, e.g., 1-deoxythreosone or threosone.^{7,8} These short-chain α -dicarbonyls are highly reactive species, and they are essential for color formation during the Maillard reaction.

3-Deoxy-D-erythro-hexos-2-ulose can be directly generated from D-glucose or from the Amadori compound via 1,2enolization. The formation and reactivity of this α -dicarbonyl compound is well-known in Maillard literature. Hodge already postulated 3-deoxy-D-erythro-hexos-2-ulose as being involved in the formation of HMF (5-(hydroxymethyl)furfural),¹ an important product from, for example, heated honey.9,10 Another reaction pathway of 3-deoxy-D-erythro-hexos-2-ulose delivers high molecular weight, colored melanoindins. Via retroaldol reactions, the α -dicarbonyl compound is converted to methylglyoxal and subsequently to brown polymers.¹¹ Caemmerer et al.⁵ postulated a mechanism whereby deoxyosones react with each other in an aldol-type condensation to form a basic melanoidin skeleton of amino-branched sugar degradation products.¹² Both postulated melanoidin models are key products for coloration.

The aim of this work was to find out more about the formation of color in 3-deoxy-D-*erythro*-hexos-2-ulose and methylglyoxal model systems. We wanted to show that shorter-chained α -dicarbonyl compounds are responsible for a greater part in the colorization than the C6- α -dicarbonyl

compounds. Furthermore, we intended to expand knowledge of the formation of methylglyoxal-based melanoidines and whether methylglyoxal polymers take part in the formation of color.

MATERIALS AND METHODS

Chemicals. The following chemicals were obtained commercially. D-Glucose, diacetone-D-glucose, *o*-phenylenediamine, and acetic acid were purchased from Fluka (Neu-Ulm, Germany). L-Alanine, 5-(hydroxymethyl)furfural, and tributyltin hydride were purchased from Aldrich (Steinheim, Germany). Methylglyoxal (40% in H_2O), imidazole, sodium hydride (60% in mineral oil), methyl iodide, Dowex 50WX8, and 2-deoxy-D-ribose were purchased from Acros (Geel, Belgium). Pyridine, acetic anhydride, chlorosuccinic acid, decyl chloroformate, carbon disulfide, PROD (250 UN), and catalase were purchased from Sigma (Steinheim, Germany). Methanol, diethyl ether, and ethyl acetate were purchased from Roth (Karlsruhe, Germany). The quinoxalines of the standard HPLC mix were synthesized as described by Pfeifer et al.¹⁴

Synthesis of 3-Deoxy-D-*erythro*-2-hexosulose (**3-DH**).¹³ 3-Deoxy-D-*erythro*-2-hexosulose (**3-DH**) was synthesized largely according to the method of Voziyan et al.

1,2:5,6-Di-O-isopropylidene-3-O-(methylthio)thiocarbonyl-α-Dglucopyranose. To a solution of 1,2:5,6-di-O-isopropylidene-α-Dglucofuranose (5.20 g, 20.0 mmol) in 100 mL of absol THF were added imidazole (30 mg) and then NaH (1.00 g, 40 mmol). The mixture was vigorously stirred for 1 h under Ar atmosphere at ambient temperature. Carbon disulfide (6.00 mL, 100 mmol) was added, and stirring was continued for 2 h. Methyl iodide (3.00 mL, 48 mmol) was then added, and the reaction mixture was stirred for an additional 1 h. Then the reaction was quenched with 1 M HCl, and the organic layer was washed with saturated NaHCO₃ and brine. The solution was dried with Na₂SO₄ and concentrated in vacuo to give the xanthate (6.01 g, 86%) as a yellow oil. TLC R_f 0.86 (EtOAc); ¹H NMR (400 MHz, CDCl₃): δ [ppm] 1.41 (s, 12H), 2.00 (s, 3H), 3.83 (t, *J* = 2.8 Hz, 1H), 3.89 (m, 1H), 3.98 (m, 1H), 4.14 (m, 1H), 4.21 (m, 1H), 4.29 (m,

Received:	July 11, 2012
Revised:	February 19, 2013
Accepted:	February 23, 2013
Published:	February 23, 2013

Journal of Agricultural and Food Chemistry

1H), 5.82 (d, *J* = 3.6 Hz, 1H); 13 C NMR (100 MHz, CDCl₃): δ [ppm] 19.9, 26.6, 65.9, 73.1, 74.4, 76.6, 81.4, 105.6, 113.5, 215.3.

1,2:5,6-Di-O-isopropylidene-3-deoxy-α-D-glucofuranose. Tributyltin hydride (4.00 mL, 13.7 mmol) was dissolved in 50 mL absol toluene and refluxed. Xanthate (3.50 g, 10 mmol) dissolved in 60 mL of toluene was added by a dropping funnel. Refluxing was continued overnight, and the solution was concentrated in vacuo. Acetonitrile (40 mL) and petroleum ether (40 mL) were added and stirred vigorously for 15 min. The two phases were separated, and the petroleum ether phase was washed with acetonitrile twice. The combined acetonitrile phase was evaporated. Purification via flash chromatography on silica (EtOAc:petroleum ether, 1:1) afforded pure 1,2:5,6-di-O-isopropylidene-3-deoxy-α-D-glucofuranose (2.11 g) in 67% yield. TLC *R*_f 0.75 (EtOAc); ¹H NMR (400 MHz, CDCl₃): δ [ppm] 1.41 (s, 12H), 1.80 (m, 1H), 2.04 (m, 1H), 3.82 (t, *J* = 2.8 Hz, 1H), 4.11–4.14 (m, 3H), 4.76 (t, *J* = 8.4 Hz, 1H), 5.82 (d, *J* = 3.6 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ [ppm] 26.7, 35.2, 67.2, 78.6, 80.4, 105.6, 109.6, 111.3.

3-Deoxy-D-glucose. 1,2:5,6-Di-O-isopropylidene-3-deoxy- α -D-glucofuranose (1.50 g, 6.14 mmol) was dissolved in 120 mL of 0.1% H₂SO₄, and the reaction mixture was refluxed for 1 h. After cooling, the solution was treated with Dowex 1X8 (HCO₃⁻) to adjust the pH to 7. The mixture was filtered through a folded filter and concentrated in vacuo to give 3-deoxy-D-glucose (1.01 g) in quantitative yield. TLC R_f 0.05 (EtOAc).

3-Deoxy-D-erythro-hexos-2-ulose. 3-Deoxy-D-glucose (500 mg, 3.04 mmol) was dissolved in 500 mL of double distilled H₂O and stirred with aeration at 25 °C. PROD (250 IU) and catalase (10 000 IU, 0.2 mg, Sigma) were added while the pH was maintained at 7.0 with periodic additions of 0.01 N NaOH. The reaction was completed after 5 h. The enzyme was filtered off through a membrane filter (0.2 μ m filter) and rinsed with H2O. The solvent was removed at 30 °C in vacuo. Purification via ionic exchange chromatography on Dowex 50X8 (200-400 mesh) in the Ca²⁺ form afforded pure 3-deoxy-Derythro-hexos-2-ulose (492 mg) in 99% yield. TLC $R_{\rm f}$ 0.2 (EtOAc); ¹H NMR (400 MHz, D_2O): δ [ppm] 1.99 (s, 0.2H), 2.35–2.43 (q, J = 6Hz, 2.5H), 2.58-2.59 (m, 1H), 2.62-2.64 (m, 1.5H), 2.67-2.68 (d, J = 3.6 Hz, 0.5H), 3.05 (d, J = 7.2 Hz, 0.3H), 3.60–3.63 (m, 2.5H), 3.75-3.78 (m, 2H), 3.84-3.87 (m, 2H), 4.02-4.06 (m, 2.5H), 4.26-4.29 (m, 1.5H), 4.41 (d, J = 2.8 Hz, 1.5H), 4.55-4.60 (m, 0.5H), 5.03–5.05 (m, 0.5H), 5.19 (s, 0.1H), 5.3 (q, J = 9.6 Hz, 0.3H), 8.22 (s, 1H), 8.29 (s, 0.2H), 8.45 (s, 1H). ¹³C NMR (100 MHz, D_2O) δ [ppm] 40.1, 40.3, 61.2, 62.7, 65.5, 69.3, 69.7, 71.9, 74.6, 164.3, 171.1, 179.5. GC/MS (3-DH-quinoxaline after acetylation): $t_{\rm R}$ 27.99 min; m/z 360 (0.2%, M⁺), 301 (7), 300 (9), 258 (12), 241 (49), 199 (33), 181 (24), 157 (34), 144 (100), 102 (11), 43 (89).

D-Glucose Model Reactions. In a typical experiment an aqueous solution of D-glucose (180 mg, 1 mmol) with or without L-alanine (89 mg, 1 mmol) in 10 mL of H₂O or phosphate buffer (pH 5) was adjusted to a starting pH of 5 with 3 N HCl or to a pH of 8 with 3 N NaOH. The model solutions were heated in sealed ampules at 100 ± 1 °C or at 130 ± 1 °C for up to 300 min in a thermoblock (Behr Labor Technik, behrotest ET2). After a defined reaction time, 500 µL of the samples was trapped with 500 µL of 0.05 M *o*-phenylenediamine solution to intercept the α-dicarbonyl compounds as quinoxalines. After 3 h at 25 °C, quinoxalines were analyzed by HPLC-DAD and GC/MS after acetylation.

α-Dicarbonyl Model Reactions. In a typical experiment an aqueous solution of an α-dicarbonyl compound (methylglyoxal, 3-deoxy-*D*-*erythro*-hexos-2-ulose, 0.1 mmol) with or without L-alanine (89 mg, 1 mmol) in 10 mL of H₂O or phosphate buffer (pH 5) was adjusted to a pH of 5 with 3 N HCl or to a pH of 8 with 3 N NaOH. The model solutions were heated in sealed ampules at 100 ± 1 °C or at 130 ± 1 °C for up to 300 min in a thermoblock (Behr Labor Technik, behrotest ET2). After a defined reaction time, 500 μL of the samples was trapped with 500 μL of 0.05 M *o*-phenylenediamine solution to intercept the α-dicarbonyl compounds as quinoxalines. After 3 h at 25 °C, quinoxalines were analyzed by HPLC-DAD and GC/MS after acetylation.

Deaerated Incubations. Degradation of α -dicarbonyl compounds under deaerated conditions was carried out using bidistilled water.

Water was degassed with helium before samples were prepared: samples were deaerated with argon before thermal treatment.

Derivatization Reactions. *Quinoxaline Derivatives.* The method described in ref 15 was adopted: $500 \ \mu$ L of the sample was spiked with $500 \ \mu$ L of *o*-phenylendiamine (0.05 M) and kept for 3 h at rt prior to injection into the HPLC-DAD system. Quinoxalines were monitored at $\lambda_{\rm M} = 320$ nm. Quantification was carried out by comparison of peak areas with those of standard solutions containing amounts of pure, authentic reference compounds. The standard solution contains commercially available quinoxalines such as quinoxaline, 2-methyl-quinoxaline, and 2,3-dimethylquinoxaline and pure authentic quinoxalines synthesized in our working group. With this quinoxaline method, acids and imidazoles can be identified as well.

Acetylated Derivatives. The method described in ref 16 was adopted: Samples were extracted twice with *n*-BuOH. The combined organic layers were spiked with 5 μ L of internal standard (5.5 mg of diphenylquinoxalin/10 mL of toluene), and the solvent was dried. A 400 μ L amount of toluene/pyridine (30:1) and 100 μ L of acetic anhydride were added to the residue and incubated for 30 min at 70 °C. The samples were analyzed by GC/MS in selected ion mode (SIM). Quantification was carried out by comparison of peak areas obtained in the TIC with those of standard solutions containing amounts of pure, authentic reference compounds.

Decyl Chloroformate Derivative of Acetic Acid. The method described in ref 17 was adopted: Samples ($60 \ \mu$ L) were spiked with a solution of chlorosuccinic acid ($50 \ \mu$ g) in water as internal standard, and $40 \ \mu$ L of pyridine and $50 \ \mu$ L of decyl chloroformate were added. The mixture was sonicated for 10 min, and afterward the decyl esters were extracted with 200 μ L of hexane. The organic layer was analyzed by GC-FID. Quantitative results were obtained by internal calibration using commercially available acetic acid. (Data obtained with GC-FID showed standard deviations <20 mmol/mol methylglyoxal and resulting coefficients of variation <3.5%. Coefficient of determination was always >0.97).

Chromatography. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Preparative column chromatography was performed on silica gel 60, 40–63 μ m (Merck, Darmstadt, Germany). All solvents were of chromatographic grade.

Nuclear Magnetic Resonance Spectroscopy (NMR). NMR spectra were recorded on a Bruker AC 400 instrument (Rheinstetten, Germany). Chemical shifts are given in parts per million relative to residual nondeuterated solvent as an internal reference.

High Performance Liquid Chromatography (HPLC/DAD). Instrumentation: Degasser, Gegasys DG-13000 (Knauer); pump, Shimadzu LC-10 AT; thermostat, 30 °C, Shimadzu CT0-6A; guard column, Nucleosil 120-5 C18 Macherey-Nagel; column, Nucleosil 5 C18 (250 mm × 4.6 mm); injection volume, 40 μ L; eluent, methanol/ water gradient, 1 mL/min.

High Performance Liquid Chromatography (HPLC/MS). Instrumentation. Degasser: Agilent 1100; pump: Agilent 1100; thermostat: 30 °C; guard column: Nucleosil 120-5 C18 Macherey-Nagel; column: Nucleosil 5 C18 (250 mm × 4.6 mm); injection volume: 40 μ L; eluent: methanol/water gradient, 1 mL/min; detector: Quattro LC, Waters.

Gas Chromatography (GC/MS). Gas chromatograph: Finnigan GCQ; capillary column: BPX 5 (SGE, 30 m, 0.25 mm ID, 0.5 μ m film thickness); carrier gas: helium 4.6; detector: Finnigan ion trap mass analyzer GCQ; injection temperature: 270 °C; temperature program: initial temperature 95 °C, hold 1 min, 95 °C to 200 °C at 15 °C/min, 200 °C for 1 min, 200 °C to 280 °C at 3 °C/min, 280 °C for 5 min, 280 °C to 300 °C at 5 °C/min, 300 °C for 5 min.

Gas chromatography (GC/MS) was used for the oligomeric compounds of MGO. Gas chromatograph: Agilent 5979; mode: electron ionization (EI), 70 eV; capillary column: fused silica capillary column Optima-5, (30 m, ID 25 mm, FD 1.0 μ m Macherey-Nagel); carrier gas: helium 4.6; injection temperature: 220 °C; temperature program: initial temperature 50 °C isotherm, 50 °C to 300 °C at 120 °C/min, 300 °C for 2.4 min.



Figure 1. Degradation of 3-deoxy-D-*erythro*-hexos-2-ulose (3-DH) resulting in formation of 3,4-dideoxyglucoson-3-ene (3,4-DDH), methylglyoxal (MGO), 5-(hydroxymethyl)furfural (HMF), and diacetyl (DA) in model systems thermally treated at 130 $^{\circ}$ C at a pH of 5 over a 300 min time period measured as quinoxalines via HPLC.



Figure 2. Degradation of 3-deoxy-D-*erythro*-hexos-2-ulose (3-DH) resulting in formation of 2-ribonic acid, 3-deoxyhexonic acid, pyruvate, methylglyoxal (MGO), and diacetyl (DA) in model systems thermally treated at 130 $^{\circ}$ C at a pH of 8 over a 300 min time period measured as quinoxalines via HPLC.

Gas Chromatography (GC/FID). Gas chromatograph: HP 6890N chromatograph equipped with a flame ionization detector. Capillary column: BPX 5 (SGE, 30 m, 0.25 mm ID, 0.5 μ m film thickness); carrier gas: helium 4.6; injection temperature: 250 °C; split ratio: 1:10; detector: 270 °C.

RESULTS AND DISCUSSION

The degradation of 3-deoxy-D-*erythro*-hexos-2-ulose and methylglyoxal in the absence or presence of an amino acid in model systems, which were thermally treated at 130 °C at a pH of 5 or a pH of 8 for a defined time, was studied.

3-Deoxy-D-*erythro***-hexos-2-ulose Model Systems.** The degradation of 3-deoxy-D-*erythro*-hexos-2-ulose leads to several peaks, which could be qualified and quantified as quinoxalines via HPLC/MS or acetylated via GC/MS. Important signals that

could be identified were assigned to *o*-phenylendiamine, 3deoxy-D-*erythro*-hexos-2-ulose, 3,4-dideoxyglucoson-3-ene, methylglyoxal, diacetyl, and 5-(hydroxymethyl)furfural. As excepted *o*-phenylenediamine applied as derivatization reagent in large excess gave the highest signal. The degradation of 3deoxy-D-*erythro*-hexos-2-ulose (at a pH of 5) and the formation of generated products are shown in Figure 1. Apparently, 3deoxy-D-*erythro*-hexos-2-ulose degradation proceeded quite fast. After 30 min of thermal treatment at 130 °C, nearly 50% of the α -dicarbonyl compound was decomposed, and after 180 min, it could not be detected anymore. Obviously, 5-(hydroxymethyl)furfural was formed rather fast within the first 30 min; afterward, the maximum was exceeded and the 5-(hydroxymethyl)furfural concentration decreased. As minor



Figure 3. Degradation of methylglyoxal (MGO) resulting in formation of 3-deoxytetrosulose (3-DT) and diacetyl (DA) in model systems thermally treated at 130 $^{\circ}$ C at a pH of 5 over a 300 min time period measured as quinoxalines via HPLC.



Figure 4. Degradation of methylglyoxal (MGO) resulting in formation of acetic acid and diacetyl (DA) in model systems thermally treated at 130 $^{\circ}$ C at a pH of 8 over a 300 min time period measured as quinoxalines via HPLC.

compounds, 3,4-dideoxyglucoson-3-ene, methylglyoxal, and later diacteyl could be identified. 3,4-Dideoxyglucoson-3-ene is a precursor of 5-(hydroxymethyl)furfural and was generated within the first 60 min and subsequently converted into 5-(hydroxymethyl)furfural. Methylglyoxal and diacetyl could be detected but only in very low concentrations.

Figure 2 shows the degradation of 3-deoxy-D-*erythro*-hexos-2ulose at an alkaline pH value. In comparison to slightly acidic reaction conditions, the observed product is completely different. Important signals that could be identified were assigned to 3-deoxy-D-*erythro*-hexos-2-ulose, 2-deoxyribonic acid, 4,5,6-trihydroxy-2-oxohexanoic acid (in the following referred to as 3-deoxyhexonic acid), pyruvate, methylglyoxal, and diacetyl. What is remarkable here are the very high amounts of 2-deoxyribonic acid, which could already be detected in the unheated standard. 3-Deoxy-D-*erythro*-hexos-2ulose underwent oxidative cleavage at room temperature and yielded formic acid and 2-deoxyribonic acid. After thermal treatment at 130 °C for 30 min, 3-deoxy-D-*erythro*-hexos-2ulose could not be detected anymore. A further degradation product of 3-deoxy-D-*erythro*-hexos-2-ulose, which was generated in relatively high amounts, was 3-deoxyhexonic acid. Obviously, this compound was formed rather slowly and did not pass a maximum concentration after 300 min of thermal treatment. Short-chain compounds such as methylglyoxal, pyruvate, an oxidation product of methylglyoxal, and diacetyl could be detected but only as minor decomposition products.

Article

The Maillard model system 3-deoxy-D-*erythro*-hexos-2-ulose/ L-alanine (not shown within this work) afforded a quite similar product spectrum. The only difference was that no 5-(hydroxymethyl)furfural could be detected but rather the pyrrole derivative. In all 3-deoxy-D-*erythro*-hexos-2-ulose model systems, but especially in the alkaline model systems, formaldehyde could be detected as a precursor for the oxidative formic acid formation.

Journal of Agricultural and Food Chemistry

Methylglyoxal Model Systems. To compare the reaction behavior of 3-deoxy-D-erythro-hexos-2-ulose, a α -dicarbonyl compound, which is stabilized by formation of many isomers, the same experiments were accomplished with methylglyoxal, a short-chain α -dicarbonyl compound. Figure 3 shows the thermal treatment at 130 °C at a pH of 5 of a methylglyoxal model system under aerated conditions. Important signals that could be identified were assigned to methylglyoxal, 3deoxytetrosulose, and diacetyl. Obviously, methylglyoxal degradation was relative slow; after a thermal treatment at 130 °C for a 300 min time period, the α -dicarbonyl compound still could be measured in concentrations of 20% of the starting value. 3-Deoxytetrosulose could be detected as a α -dicarbonyl compound, too. This product could be generated from methylglyoxal and formaldehyde. Formaldehyde was formed in high amounts due to a α -dicarbonyl cleavage of methylglyoxal. The LC/MS experiments as well as HPTLC measurements (data not shown) showed that most of the decomposed methylglyoxal is converted to a dimer of methylglyoxal. This compound was generated in concentrations almost up to 50% of the methylglyoxal starting value.

At alkaline pH values, the concentration of methylglyoxal decreased relatively fast (see Figure 4). The thermal treatment of methylglyoxal at 130 °C at a pH of 8 afforded a completely different product spectrum in comparison to experiments at a pH of 5. As a main degradation product, acetic acid could be identified. Acetic acid is an autoxidation product and could not be measured under deaerated conditions. Another α -dicarbonyl compound, which could be analyzed as a minor degradation product, was diacetyl.

In Maillard model system methylglyoxal/L-alanine the degradation of proceeded faster. At slightly acidic pH values a quite similar product spectrum could be observed, and at alkaline pH values, the amino acid had influence on the generated products. Whereas in pure methylglyoxal model systems acetic acid is one of the main degradation products, in the Maillard model systems with L-alanine, pyruvate could be measured in high amounts (e.g., after a thermal treatment over a 60 min time period, 60% of the starting value was converted to pyruvate). On the basis of all these results, the product formation during the degradation of 3-deoxy-D-erythro-hexos-2ulose depends on the adjusted pH value. Whereas at slightly acidic pH values one of the main degradation products was 5-(hydroxymethyl)furfural, at alkaline pH values the formation of autoxidation products such as 2-deoxyribonic acid and 3deoxyhexonic acid was preferred. Methylglyoxal yielded at pH 5 as main components the aldehydes formaldehyde and acetaldehyde and a dimer of methylglyoxal. At alkaline pH values, again autoxidation products such as acetic acid and pyruvat are generated predominantly.

Although mechanistic studies of the HMF formation from Dglucose, respectively, 3-deoxy-D-*erythro*-hexos-2-ulose, are well investigated, we could show in our work that 3-deoxy-D-*erythro*hexos-2-ulose did not produce colored products such as in Maillard literature-postulated melanoidines⁵ but preferentially acidic cleavage products. Thermal treatment of a 3-deoxy-D*erythro*-hexos-2-ulose solution in the absence or presence of an amino acid at 130 °C over a 300 min time period did not embrown the solution, and the maximal absorption (measured at 420 nm) was 0.1 (control: double distilled water).

At alkaline pH values, the product spectrum derived from 3deoxy-D-*erythro*-hexos-2-ulose is more complex. Main products are the autoxidation products 2-deoxyribonic acid and 3deoxyhexonic acid (Figure 5). As minor components, the autoxidation product pyruvate and the α -dicarbonyl com-



Figure 5. Alkaline degradation pathway of 3-deoxy-D-*erythro*-hexos-2ulose (3-DH) via oxidative processes yielded 2-deoxyribonc acid, 3deoxyhexonic acid, and pyruvate.

pounds methylglyoxal and diacetyl could be detected. These α dicarbonyl compounds are typical degradation products reported in Maillard literature. Yaylayan and co-workers^{18,19} found that a C₃/C₄-cleavage of 3-deoxy-D-*erythro*-hexos-2-ulose produced methylglyoxal and glyceraldehyde that subsequently underwent amino acid-mediated chain elongation to form diacetyl.

2-Deoxyribonic acid was generated via an oxidative α dicarbonyl cleavage. Direct autoxidation of 3-deoxy-D-*erythro*hexos-2-ulose afforded the oxidation product 3-deoxyhexonic acid. One explanation for the very high amounts of 2deoxyribonic acid could be the rise of entropy (Figure 5). Due to the formation of various small molecule acids at alkaline pH values as main degradation products of 3-deoxy-D-*erythro*hexos-2-ulose, no coloration of the reaction solution could be detected.

Having demonstrated that color formation is not based on 3deoxy-D-*erythro*-hexos-2-ulose in Maillard reaction systems, we turned our focus on the investigation of methylglyoxal-derived melanoidins. Thermal treatment of methylglyoxal model solutions is well investigated in Maillard literature. Fiedler and co-workers¹¹ postulated a high molecular weight melanoidin skeleton based on methylglyoxal units (Figure 6). Due to the conjugated melanoidin structure derived from methylglyoxal polymerization, the model solutions are highly embrowned. The aldol condensation reactions which lead to the melanoidins proceed very efficiently at alkaline pH values, which explains the even more intensive coloration at pH 8.

Our investigations showed that methylglyoxal reacts under slightly acidic conditions via two main degradation pathways (Figure 6). Short-chained reactive products are generated via an aldol reaction of methylglyoxal with formaldehyde to 3deoxytetrosulose or by dimerization of acetaldehyde and a subsequent oxidation to yield diacetyl. The other pathway leads to high molecular weight, brownish compounds via a dimer, which affords in further aldol condensation the melanoidin



Figure 6. Main degradation pathways of thermally treated methylglyoxal under slightly acidic conditions yielded short-chain α -dicarbonyl compounds such as 3-deoxytetrosulose (3-DT), diacetyl, and high molecular weight melanoidins.



Figure 7. The GC/MS chromatogram of MGO and its polymers: the dimer at 2.67 min and the trimer at 3.45 min.

postulated by Fiedler.¹¹ The dimer could be identified by LC/ MS for the first time, was further investigated by GC/MS, and is a good choice for the postulated melanoidin structures. Therefore, an aqueous solution of methylglyoxal was stored for seven days at 0 °C and subsequently measured and interpreted by GC/MS without derivatization. Two of the main resulting products were a dimer of methylglyoxal with a mass/charge ratio of 146.06 g/mol $[m/z = 145 \rightarrow 115 \rightarrow 73 \rightarrow 59 \rightarrow 43]$ and a trimer with a mass/charge ratio of 216.06 g/mol $[m/z = 215 \rightarrow 187 \rightarrow 159 \rightarrow 115 \rightarrow 73 \rightarrow 59 \rightarrow 43]$ (MS fragmentation in the negative mode in square brackets) (Figure 7).

Thermal treatment of the Maillard model system methylglyoxal/L-alanine leads to deeper embrowned solutions, e.g., thermal treatment at 130 $^{\circ}$ C over a 300 min time period at a pH of 5 gave an absorption of 0.8 (measured at 420 nm, dilution factor 100) in the absence of L-alanine and an absorption of 1.3 in the presence of L-alanine. An explanation for this could be the formation of the melanoidin structure postulated by Tressl and co-workers (Figure 6).²⁰

At alkaline pH values, the product spectrum derived from methylglyoxal showed the same phenomenon of preferred oxidation processes as already observed for 3-deoxy-D-*erythro*hexos-2-ulose model systems. Under caramelization conditions, methylglyoxal reacted preferentially via an oxidative α dicarbonyl cleavage to formic acid and acetic acid. Under Maillard conditions, thermal treatment of methylglyoxal yielded pyruvate as one main product. Because of the relatively high amounts of generated acids, the pH value decreased, and after a short reaction time, the conditions were approaching those of the pH 5 models. The increased colorization at alkaline pH values could be explained with the preferred aldol reactions under basic conditions. In contrast to acidic pH values the conjugated melanoidins were built up faster and furnished deep brown model solutions.

We showed that 3-deoxy-D-*erythro*-hexos-2-ulose and its thermally formed degradation products are not directly involved in coloration during the Maillard reaction. Main degradation products are 5-(hydroxymethyl)furfural and subsequently formed levulinic acid in the absence of amino acids and pyrrole derivatives in presence of amino acids. Furthermore, we validated the formation of brownish melanoidins based on methylglyoxal units by LC/MS investigations. Our results emphasize that color in Maillard systems is based on short-chained α -dicarbonyl compounds and its degradation products rather than on those with a C₆-frame.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Hodge, J. E. Chemistry of Browning Reactions in Model Systems. *J. Agric. Food Chem.* **1953**, *1*, 928–943.

(2) Maillard, L. C. C. R. Acad. Sci. Ser. 1912, 154, 1554.

(3) Ledl, F.; Schleicher, E. Die Maillard-Reaktion in Lebensmitteln und im menschlichen Körper - neue Ergebnisse zu Chemie, Biochemie und Medizin. *Angew. Chem.* **1990**, *102*, 597–626.

(4) Perez-Martinez, M.; Caemmerer, B.; Pena, M. D.; Kroh, L. W. Influence of Brewing Method and Activity Regulators on the Antioxidant Capacity of Coffee Brew. J. Agric. Food Chem. 2010, 58, 2958–2965.

(5) Caemmerer, B.; Kroh, L. W. Intact Carbohydrate Structures as Part of the Melanoidin Skeleton. *J. Agric. Food Chem.* **2002**, *50*, 2083–2087.

(6) 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.

(7) Voigt, M.; Glomb, M. A. Reactivity of 1-Deoxy-D-*erythro*-hexo-2,3-diulose: A Key Intermediate in the Maillard Chemistry of Hexoses. *J. Agric. Food Chem.* **2009**, *57*, 4765–4770.

(8) Voigt, M.; Smuda, M.; Pfahler, C.; Glomb, M. A. Oxygen-Dependent Fragmentation Reactions during the Degradation of 1-Deoxy-D-*erythro*-hexo-2,3-diulose. *J. Agric. Food Chem.* **2010**, *58*, 5685–5691.

(9) Anet, E. F. L. J. 3-Deoxyglycosuloses (3-deoxyglycosones) and the Degradation of Carbohydrates. *Adv. Carbohydr. Chem* **1964**, *19*, 181–218.

(10) Fallico, B.; Arena, E.; Zappala, M. Degradation of 5-Hydroxymethylfurfural in Honey. *J. Food Sci.* **2008**, *73*, C625–C631. (11) Fiedler, T.; Moritz, T.; Kroh, L. W. Influence of alpha-Dicarbonyl Compounds to the Molecular Weight Distribution of Melanoidins in Sucrose Solutions, Part 1. *Eur. Food Res. Technol.* **2006**, *223*, 837–842.

(12) Caemmerer, B.; Kroh, L. W. Investigation on the Influence of Reaction Conditions on the Elementary Composition of Melanoidins. *Food Chem.* **1995**, *53*, 55–59.

(13) Chetyrkin, S. V.; Zhang, W.; Hudson, B. G.; Serianni, A. S.; Voziyan, P. A. Pyridoxamine Protects Proteins from Functional Damage by 3-Deoxyglucosone: Mechanism of Action of Pyridoxamine. *Biochemistry* **2008**, *47*, 997–1006.

(14) Pfeifer, Y. V.; Kroh, L. W. Investigation of Reactive α -Dicarbonyl Compounds Generated from the Maillard Reactions of L-Methionine with Reducing Sugars via Their Stable Quinoxaline Derivatives. J. Agric. Food Chem. **2010**, 58, 8293–8299.

(15) Hollnagel, A.; Kroh, L. W. Degradation of Oligosaccharides in Nonenzymatic Browning by Formation of α -Dicarbonyl Compounds via a "Peeling Off" Mechanism. *J. Agric. Food Chem.* **2000**, *48*, 6219–6266.

(16) Nedvidek, W.; Ledl, F.; Fischer, P. Detection of 5-Hydroxymethyl-2-methyl-3(2H)-furanone and of alpha-Dicarbonyl Compounds in Reaction Mixtures of Hexoses and Pentoses with Different Amines. Z. Lebensm. Unters. Forsch. A 1992, 194, 222–228. (17) Husek, P. Amino-Acid Derivatization and Analysis in 5 Minutes. FEBS Lett. 1991, 280, 354–356.

(18) Yaylayan, V. A.; Keyhani, A. Origin of 2,3-Pentandione and 2,3-Butandione in D-Glucose/L-Alanine Maillard Model Systems. *J. Agric. Food Chem.* **1999**, 47, 3280–3284.

(19) Yaylayan, V. A.; Keyhani, A. Origin of Carbohydrate Degradation Products in L-Alanine/ $D[^{13}C]$ -Glucose Model Systems. *J. Agric. Food Chem.* **2000**, *48*, 2415–2419.

(20) Tressl, R.; Wondrak, G. T.; Garbe, L.-A.; Krüger, R.-P.; Rewicki, D. Pentoses and Hexoses as Sources of New Melanoidin-like Maillard Polymers. *J. Agric. Food Chem.* **1998**, *46*, 1765–1776.