Degradation of Oligosaccharides in Nonenzymatic Browning by Formation of α-Dicarbonyl Compounds via a "Peeling Off" Mechanism

Anke Hollnagel and Lothar W. Kroh*

Institut für Lebensmittelchemie, Technische Unversität Berlin, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany

The formation of α-dicarbonyl-containing substances and Amadori rearrangement products was studied in the glycine-catalyzed (Maillard reaction) and uncatalyzed thermal degradation of glucose, maltose, and maltotriose using *o*-phenylenediamine as trapping agent. Various degradation products, especially α-dicarbonyl compounds, are formed from carbohydrates with differing degrees of polymerization during nonenzymatic browning. The different Amadori rearrangement products, isomerization products, and α-dicarbonyls produced by the used carbohydrates were quantified throughout the observed reaction time, and the relevance of the different degradation pathways is discussed. In the Maillard reaction (MR) the amino-catalyzed rearrangement with subsequent elimination of water predominated, giving rise to hexosuloses with α-dicarbonyl structure, whereas under caramelization conditions more sugar fragments with an α-dicarbonyl moiety were formed. For the MR of oligosaccharides a mechanism is proposed in which 1,4-dideoxyosone is formed as the predominating α-dicarbonyl in the quasi-water-free thermolysis of di- and trisaccharides in the presence of glycine.

Keywords: *Nonenzymatic browning; Maillard reaction; α-dicarbonyl compound; oligosaccharides; peeling off mechanism*

INTRODUCTION

The Maillard reaction (MR) has been investigated thoroughly for monosaccharides and amino acids in aqueous solution (Ledl and Schleicher, 1990). However, most real food systems contain oligosaccharides or even polymeric saccharides; therefore, it should be of great interest to study how those carbohydrates contribute to the MR in the formation of flavor and color.

For mono- and disaccharides significant differences are observed in the spectrum of volatile compounds formed. Glucose undergoes reaction toward the dihydro-γ-pyranone or hydroxymaltol, whereas maltose, caused by the glycosidic substituent, favors pathways toward maltol or heterocyclic compounds that still possess the glycosidic substituent, such as 4-(glucopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one or 4-(glucopyranosyloxy)-5-(hydroxymethyl)-2-methyl-3(2)-furanone (Pischetsrieder and Severin, 1994). Furthermore, the position of the glycosidic link is reported to have consequences for the reactivity of disaccharides in MR (Kato et al., 1989).

Additionally it was demonstrated by a kinetic approach that di- and oligosaccharides show principally a longer induction phase in the formation of brown color (Wedzicha and Kedward, 1995). They react slower, which suggests that oligomers first have to be converted into more reactive, smaller intermediates.

Kroh et al. (1996) reported the breakdown of oligo- and polysaccharides to nonvolatile reaction products by formation of a glycosyl cation and subsequent formation of either anhydrosugars and differently linked saccharides or, in the presence of water, mainly mono- and oligosaccharides. Parameters such as water content obviously play an important role (Häseler and Kroh, 1998) and have not been considered thoroughly in the investigations of oligosaccharides until now.

Whether degradation of the oligosaccharides also occurs via interaction of the amino compound with the free glycosidic OH group is currently under investigation. Pischetsrieder et al. (1998) published the formation of amino reductones in aqueous solution from disaccharides with various amino compounds. It is therefore reasonable to assume that reaction of the free glycosidic OH group can initialize the breakdown of the sugar molecule.

In the course of MR, compounds with α-dicarbonyl structure are reported to play a key role in the initial and advanced stages (Beck et al., 1988). Those α-dicarbonyls are very reactive, which explains their importance throughout the reaction, but also complicates their quantification. Although they have been investigated in mixtures containing monosaccharides (Nedvidek et al., 1992), it is not known whether the different behavior of oligosaccharides in MR is caused by differences in the formation of α-dicarbonyls.

In our research we investigated the formation of α-dicarbonyls, as nonvolatile key intermediates in caramelization and the Maillard reaction, throughout the thermolysis of saccharides with different degrees of polymerization (dp) using glycine in the case of the Maillard reaction, and in the presence of the trapping reagent *o*-phenylenediamine (OPD).

* To whom correspondence should be addressed. Phone: +49-30-31472584. Fax: +49-30-31472585. E-mail: lothar.kroh@tu-berlin.de.

10.1021/jf9906127 CCC: $19.00
Published on Web 10/28/2000

EXPERIMENTAL PROCEDURES

Thermal Treatment. *Caramelization*. Mono-, di-, or trisaccharide and *o*-phenylenediamine (OPD), 1 mmol of glucose (Glc) (Merck, water-free), maltose monohydrate (Mal) (Merck), or maltotriose (Fluka, >93% HPLC), and 1 mmol of OPD (recrystallized from water), are mixed thoroughly by use of a vortex and heated to 480 min at 100 ± 1 °C in sealed tubes by means of a thermoblock (behrotest ET 1, behr Labor Technik).

Maillard Reaction. Mono-, di-, or trisaccharide, glycine, and OPD, 1 mmol of glucose, maltose monohydrate, or maltotriose, 1 mmol of glycine (Serva), and 1 mmol of OPD, are mixed thoroughly and heated for up to 480 min at 100 ± 1 °C in sealed tubes.

For determination of furosine, 1 mmol of glucose or maltose was mixed thoroughly with 1 mmol of Boc-lysine and heated to 90 min at 120 ± 1 °C in sealed tubes. The reaction was carried out in distilled water; pH was not controlled.

After thermolysis samples were dissolved in 10 mL of a mixture of methanol (Merck, suprasolv) and distilled water, for furosine determination only in water, filtered, and diluted if necessary. Experiments were carried out in duplicate; where necessary, additional experiments were conducted to obtain reliable results.

Synthesis of Quinoxalines. *Synthesis of 2-methyl-3-(1,2,3-trihydroxypropyl)quinoxaline* was performed according to Beck and Ledl (1988). The Amadori rearrangement product 1-deoxy-1-piperidino-D-fructose was obtained according to Hodge and Fisher (1963).

The fructosylpiperidine was mixed with twice as much OPD, solved in phosphate buffer (pH 7, 10 mL), boiled under reflux, and then concentrated. The resulting fine precipitate was soluble under heating, and with slow cooling good crystallization was achieved. Repeated recrystallization from acetone gave a yellow-white precipitate. Its identity was proven by various spectrometric measures. ^{1}H NMR (300 MHz) in CD_3OD (Aldrich): δ 2.85 (s, 3H), 3.85 (dd, 1H), 3.93 (dd, 1H), 5.95 (d, 1H), 7.74 (m, 2H), 7.96 (m, 1H), 8.07 (m, 1H). Mass spectrum of the acetylated substance: *m*/*z* 360 (0.2, M^+), 301 (4), 300 (4), 259 (31), 258 (27), 241 (18), 174 (100), 157 (5), 107 (22) 188 (3), 103 (9), 43 (96).

Synthesis of 2-(2,3,4-trihydroxybutyl)quinoxaline was carried out according to Madson and Feather (1981). Via the formation of the bishydrazone of 3-deoxyhexosulose free 3-deoxyhexosulose was obtained.

To the crude reaction mixture was added an approximately equimolar amount of OPD; further workup, similar to the one described for the 2-methyl-3-(1,2,3-trihydroxypropyl)quinoxaline, again gave crystals which allowed structure analysis by means of ^{1}H NMR and GC/MS. ^{1}H NMR (300 MHz) in CD_3OD: δ 3.13 (dd, 1H), 3.42 (dd, 1H), 3.60 (m, 1H), 3.66 (dd, 2H), 3.80 (dd, 1H), 7.77 (m, 2H), 8.03 (m, 2H), 8.83 (M, 1H). Mass spectrum of the acetylated substance: *m*/*z* 360 (0.2, M^+), 301 (7), 300 (9), 258 (12), 241 (14), 215 (14), 199 (33), 181 (24), 157 (34), 144 (100), 102 (11), 43 (89).

Synthesis of 2-(arabino-1,2,3,4-tetrahydroxybutyl)quinoxaline was performed according to Morita et al. (1981). ^{1}H NMR (300 MHz) in d_6-DMSO (Aldrich): δ 3.34 (s, 4H), 3.66 (dd, 1H), 4.65 (m, 1H), 5.14 (d, 1H), 5.59 (d, 1H), 7.80 (m, 2H), 8.05 (m, 2H), 9.08 (s, 1H);. Mass spectrum of the acetylated substance: *m*/*z* 418 (0.2, M^+), 358 (2), 317 (3), 316 (3), 299 (63), 257 (119, 215 (14), 202 (50), 196 (8), 171 (19), 160 (100), 143 (4), 129 (10), 115 (12) 102 (7), 43 (97).

Synthesis of 2-methyl-3-(2,3-dihydroxypropyl)quinoxaline was performed according to Morita et al. (1981). ^{1}H NMR (300 MHz) in CD_3OD: δ 2.77 (s, 3H), 3.17 (m, 2H), 3.65 (d, 2H), 4.30 (m, 1H), 7.70 (m, 2H), 7.94 (m, 2H). Mass spectrum of the acetylated substance: *m*/*z* 302 (0.2, M^+), 243 (16), 201 (42), 183 (100), 171 (40), 143 (7), 117 (9), 102 (9).

Syntheses of the Amadori rearrangement products glucosylglycine and maltosylglycine were carried out according to Kroh et al. (1992).

HPLC/DAD (Diode Array Detector). *Instrumentation*. Degasser, Degasys DG-13000 (Knauer); pump, Shimadzu LC 10 AT; thermostat, Haake F3 (Fisons); guard column, Nucleosil 120-5 C_{18} Macherey-Nagel; column, Nucleosil 5 C_{18} (250 × 4.6, 5 μm); detector, Kontron 440; flow, 1.0 mL/min; temperature, 30 °C; injection volume, 20 μL; eluent, methanol (solvent A), water (solvent B) (both HPLC grade); detection wavelength, 320 nm; full scan, 190−440 nm, gradient, 0−5 min 5% A, 5−25 min 5% → 50% A, 25−30 min 50% → 100% A, 30−40 min 100% A.

GLC/MS and GLC/FID. Extraction and derivatization were carried out according to Hollnagel and Kroh (1998).

GLC/MS−Parameters. Analytical GLC was performed on a Hewlett-Packard 5890 series II gas−liquid chromatograph equipped with a Hewlett-Packard 5989B mass spectrometer and a fused-silica capillary column, DB-5HT (J&W; i.d. 0.32 mm × 30 m, 0.1 μm film thickness) (carrier gas, He; detector/injector temperature, 280 °C; temperature program, initial temperature 120 °C, initial time 5 min isotherm, 120 → 200 °C, 10 °C/min, 5 min isotherm, 200 → 280 °C, 10 °C/min, 9 min isotherm). Column effluents were analyzed by electron-ionization mass spectrometry; to verify the identity of the analytes, they were compared with independently synthesized standards. For EI spectra, the instrument was scanned from *m*/*z* 40 amu to *m*/*z* 800 amu.

Furosine Determination (Alltech Nederland). A 2 mL reaction mixture was mixed with 6 mL of 10.6 M HCl in screw-capped vessels, the vessel was flushed with nitrogen for 2 min and then heated for 23 h at 110 °C. After heat treatment an aliquot was centrifuged, and 1 mL of the supernatant was purified by being passed through a Sep-Pack cartridge (activated with 5 mL of methanol and 10 mL of water); additional elution with 4 mL of 3 M HCl ensured that all furosine was washed from the cartridge.

HPLC Instrumentation. Pump, Spectraphysics P2000 Binary Gradient; column, furosine dedicated column (250 × 4.6, 5 μm, Alltech); detector, Spectraphysics UV2000 Dual UV/vis; flow, 1.2 mL/min; temperature, room temperature; injection volume, 20 μL; eluent, 0.4% acetic acid (v/v) (a), 0.3% KCl in A (w/v) (B) (all HPLC grade); detection wavelength, 280 nm, gradient, 0−11.5 min 100% A; 11.5−18 min 100% → 50% A, 18.5−21 min 50% A, 21−23 min 50% → 100% A, 23−32 min 100% A.

HPTLC (Kroh et al., 1996). *Monosaccharides*. HPTLC plates (Merck, Kieselgel 60, 20 × 10 cm) were used, the eluent consisted of chloroform (Merck, HPLC grade), acetic acid (Merck, 96%), methanol (Merck suprasolv), and water (60:18:12.5:5, v/v/v/v), and the plates were developed twice.

Di- and Trisaccharides. HPTLC plates were developed twice in an AMD chamber (CAMAG) using an eluent mixture of chloroform, methanol, and water (50:40:8, v/v/v) in which 2.5−2.8 mg of boric acid/98 mL of eluent mixture was dissolved. Detection was performed in both systems with diphenylamine/aniline/methanol/phosphoric acid (85%) (1 g/1 mL/100 mL/10 mL).

HPAEC/PAD (Pulsed Amperometric Detector). *Instrumentation*. Pump, Dionex GP 40; column, 2 × PA-100 (250 × 4 mm, Dionex); detector, PAD (Dionex); oven, HPLC column oven 2155 (Pharmacia); temperature, 25 °C; injection volume, 20 μL; flow, 0.5 mL/min; eluent, 0.15 N NaOH (A), 1 N NaAc in 0.15 N NaOH (B); gradient, 0−10 min 100% A, 10−60 min 0% → 60% B. Quantification was done by external calibration.

RESULTS AND DISCUSSION

A lot of investigations have been conducted on the volatile compounds that are formed in nonenzymatic browning of the various carbohydrates; however, there is evidence that also the nonvolatile fraction, especially during the initial phase of MR, contains many reactive intermediates, influencing the quality and extent of the reaction, such as color, aroma, and antioxidative activity.

Prior to the investigation of the reaction mixtures, it was confirmed that OPD inhibited the progress (browning) of the Maillard reaction. Although it is a diamine,

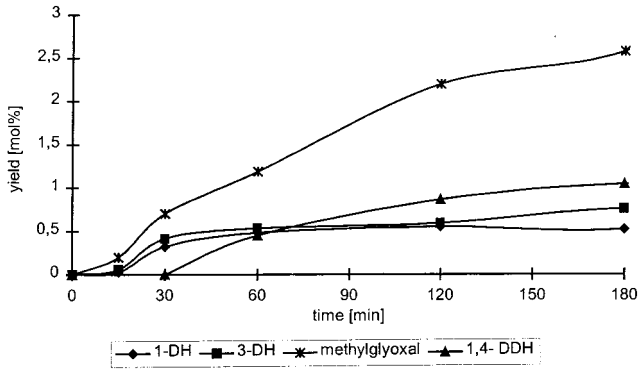

Figure 1. Formation of α-dicarbonyl compounds detected as quinoxalines in thermal treatment of Glc/OPD as a caramelization-resembling system.

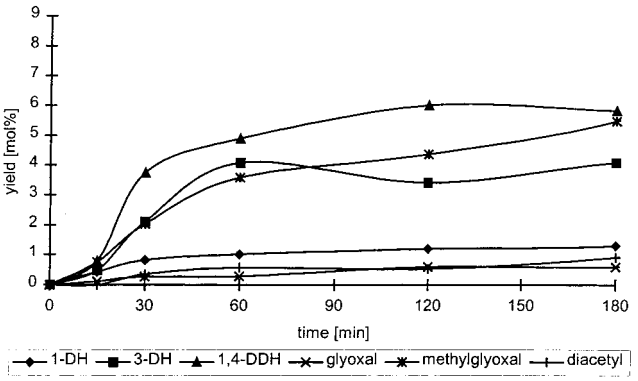

Figure 2. Formation of α-dicarbonyl compounds detected as quinoxalines in thermal treatment of Glc/Gly/OPD as a Maillard-resembling system.

it did not react disproportionately in the MR itself (Hollnagel and Kroh, 1998). It was shown that there are differences in the formation of sugar fragments with α-dicarbonyl structure between mono- and disaccharides as well as between aldoses and ketoses.

Another measure that was taken to reduce the influence of OPD on the results of the experiments was to repeat the sets of experiments with and without glycine so that the effect of the glycine could be measured independently from that of OPD.

Although questioned in recent papers (Hofmann et al., 1999), the trapping reagent was added in this investigation directly to the reaction mixture so that all formed α-dicarbonyls could be analyzed in their summed up concentration, giving a clearer insight into their relevance in the reaction mixture. Taking into account the varying reactivity of the α-dicarbonyls as reported by Glomb and Pfahler (1999), this seemed to be even more important.

With six different reference substances, determination of a wide range of α-dicarbonyl compounds quantitatively was determined. Besides quantification of α-dicarbonyls by HPLC, the identity of the various quinoxalines was confirmed by GLC/MS after derivatization.

Additionally the varying reaction mixtures were characterized with regard to the degradation of the starting carbohydrate, the formation of the Amadori rearrangement products (ARPs) and isomerization products, and, if detected, subsequent formation of carbohydrates by degradation of the starting compound.

Reactions of Monosaccharides. To determine the differences in reaction between mono- and oligosaccharides, the reaction behavior of glucose was investigated thoroughly.

Formation of α-Dicarbonyls. When the spectrum of α-dicarbonyls formed in thermal treatment from Glc/OPD and Glc/Gly/OPD was studied, there was clearly an enhancing effect of glycine (Gly) observed.

In a caramelization-resembling system, glucose alone produced only minor amounts of α-dicarbonyls. After 180 min methylglyoxal (≈2.5 mol %) was predominating and less than 1 mol % each 3-deoxyhexosulose (3-DH), 1-deoxyhexosulose (1-DH), and 1,4-dideoxyhexosulose (1,4-DDH) were formed (Figure 1).

In the MR, glycine induced the formation of α-dicarbonyls strongly. It was striking that in the presence of Gly more deoxyhexosuloses with a complete carbon chain (Σ 11 mol % at 180 min; see Figure 2) were formed than sugar fragments with an α-dicarbonyl structure (Σ 7 mol % at 180 min), which indicates that in caramelization α-dicarbonyls are rather formed via breakdown of the carbon chain (retro-aldolization), whereas in the MR they are formed by amine-catalyzed rearrangement and subsequent vinylogous *β*-elimination of water. This could also be the result of a lower pH due to the addition of the amino acid. However, measurement of the pH revealed that both reaction mixtures had similar pH values, most probably due to the OPD.

In MR systems the formation of methylglyoxal was enhanced; it was formed quickly and after 60 min had already reached a concentration of 3.6 mol %, and at 180 min was even 5.5 mol %. This phenomenon is probably based on the additional reaction pathways via cleavage of the Schiff base and 3-DH (Weenen, 1998; Hayashi et al., 1986).

Moreover, it is interesting to see that with amino catalysis besides 1-DH (1.3 mol % after 180 min) and 3-DH (4.1 mol % after 180 min) 1,4-DDH (5.8 mol % after 180 min) was also formed in comparable amounts. Nedvidek et al. (1992) attributed the formation of this substance (1,4-DDH) to Strecker degradation and showed that its formation was less with non-Strecker-active amino acids.

Measurement of the pH value after the sample of the MR mixture was dissolved in water indicated a stabile, neutral to slightly acidic reaction medium, from 6.8 to 5.5 at the end of the reaction time, due to the p*K* of OPD. This for the MR rather high pH suggests that 1-DH should play an important role (Hodge, 1967). However, in the investigated mixtures there was 3 times more 3-DH formed than 1-DH, which might be explained by the high concentration of 1,4-DDH, as the subsequent reaction product of 1-DH, as described by Nedvidek, pointing out the essential role of the α-amino acid again.

Degradation of Glucose and Isomerization to Fructose. Glucose was degraded very fast in the cases of both caramelization and the MR (Figure 3).

After 30 min more than 60 mol % glucose was converted in both cases. The fact that, in contrast to MR mixtures, in caramelization reaction mixtures 1,6-*β*-anhydroglucose was detected by HPTLC underlines the parallelism to caramelization of monosaccharides in the absence of OPD as investigated earlier (Kroh et al., 1996). In the MR no glucose could be detected after 120 min, whereas in caramelization there remained about 15 mol % starting material even after 180 min.

Under the chosen reaction conditions degradation of glucose is fast; glycine even increased the reaction rate of the degradation.

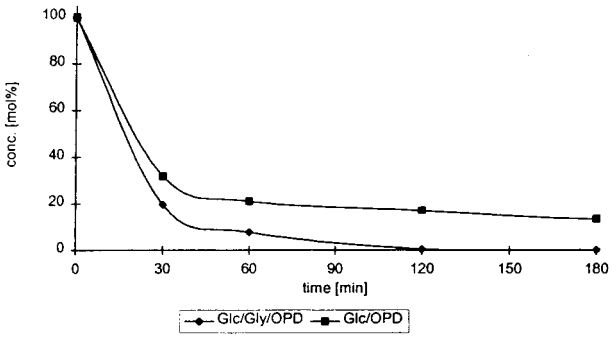

Figure 3. Degradation of glucose in caramelization (Glc/OPD) and in the MR (Glc/Gly/OPD).

Considerable formation of fructose could only be observed in reaction mixtures without glycine by means of HPTLC.

The maximal concentration observed was 6.1 mol % at 30 min, which decreased to 5 mol % after 1 h and then further to 4 mol % after 120 min and reached 3.5 mol % after 180 min. It was earlier reported that isomerization is an important reaction pathway in caramelization (Kroh et al., 1996). Although it is likely to occur in MR as well, in our case no fructose could be detected, because either the concentration of glucose decreased so fast that there was not enough starting material available to produce considerable amounts of fructose or the ketose was immediately converted.

Formation of Fructosylglycine. As fructosylglycine is reported to be an important intermediate in the MR, it was monitored as well by HPTLC. There was a maximum concentration observed; already after 30 min about 8.9 mol % Amadori rearrangement product was formed, and its concentration decreased to 7.3 mol % after 60 min when glucose had been converted virtually completely. After 120 min the concentration was as low as 2.6 mol % and decreased more slowly now to 1.9 mol % after 180 min. This result is consistent with the observation that the formation of the Amadori rearrangement product occurs in the initial phase of the MR and is readily converted further as described earlier. Therefore, it can be concluded that under the chosen conditions the typical reaction pathway for monosaccharides in the MR leads via the Amadori rearrangement product to the production of the α-dicarbonyls 3-DH, 1,4-DDH, methylglyoxal, and 1-DH.

Reaction of Disaccharides. *Formation of α-Dicarbonyls.* Under the same reaction conditions as for glucose, maltose showed a lower reactivity; therefore, the reaction time was prolonged to 240 min. In caramelization only small amounts of 1-DH and 3-DH or sugar fragments with an α-dicarbonyl moiety were detectable from the investigated dicarbonyl compounds. Except 1,4-DDH, none was formed at more than 1 mol %. 1,4-DDH was also formed (maximum 1.4 mol %, after 240 min of reaction time, Table 1).

As outlined later, the disaccharide was degraded relatively fast; after 60 min most of the maltose was degraded, indicating that formation of α-dicarbonyls plays a minor role in carbohydrate degradation under caramelization conditions.

When glycine was added, a dramatic increase in the formation of 1,4-DDH was observed. It was formed at more than 18.1 mol % at 240 min, whereas the concentration of all other detected α-dicarbonyls, among which methylglyoxal predominated (2.9 mol %, at 240 min), remained negligible (Table 1).

Table 1. Formation of α-Dicarbonyls from Maltose Detected as Quinoxalines, mol %, in Caramelization (cara; Mal/OPD) and in the Maillard reaction (MR; Mal/Gly/OPD)[a]

time,	1-DH		3-DH		1,4-DDH		glyoxal		methyl-glyoxal	
min	cara	MR	cara	MR	cara	MR	cara	MR	cara	MR
30	0.06	0.14	0.00	0.16	0.05	**0.96**	0.07	0.07	0.03	0.54
60	0.13	0.30	0.02	nr	0.15	**4.62**	0.14	0.14	0.10	1.29
120	0.08	1.43	0.20	0.96	0.54	**12.34**	0.31	0.31	0.33	2.06
180	0.15	1.84	0.26	1.44	1.06	**17.71**	0.42	0.42	0.56	2.70
240	0.17	1.89	0.42	1.35	1.44	**18.10**	0.56	0.96	0.80	2.94

[a] nr = not resolved.

Apparently the formation of 1,4-DDH is an important pathway in the degradation of maltose in the course of the Maillard reaction. We propose for its formation a pathway similar to a "peeling off mechanism" (Figure 4). This reaction principle of degradation of the oligo- or polysaccharide from the reducing end was published first by Morita et al. (1983), who investigated the degradation of differently linked homoglucans in alkaline solution. In the case of the MR, we think that glycine acts in the first instance as a catalyst in the rearrangement reactions at the reducing end of an α-glucan (**1**) via the Schiff base **2** toward the 2,3-enediol structure **3**. Either this enediol is now able to eliminate the glycosidic residue at C4, leading to 1-amino-1,4-dideoxyhexosulose (**4**) (Beck et al., 1988), or the amino residue at C1 is eliminated first. Subsequent recovery of the enediol structure **5** by Strecker degradation, for example, or by reaction with oxidized reductones then allows the elimination of the second residue, leading to the *malto*-oligosaccharide **6** and the 1,4-DDH (**7**), which was found in high amounts in the reaction mixture.

The likelihood of elimination at C4 or C1 could depend on steric properties or on electronic conditions in the molecule. Since **3** most probably does not contain charged substituents when the p*K* values (2.34 and 9.6) of glycine are considered, the steric properties should influence the further reaction. For instance, on the orientation of the π-orbitals which will form the double bond, they should be parallel for smooth elimination. Therefore, the carbon atoms where the vinylogous β-elimination will occur should lie in one plane. By molecular modeling using Alchemy II (Tripos Associates, Inc.), it was revealed that C1 of the maltose−glycine adduct lies perfectly in the plane whereas C4 lies 2.4° out of the plane. However, since the molecule is proposed to exist in the open chain form, only little energy would be necessary to accomplish this requirement for C4 as well. Furthermore, the probability of elimination depends on the size of the substituents; the bigger the substituent at C4 becomes (i.e., with increasing chain length), the more elimination at C4 should be favored.

Additional proof for the importance of the elimination of the glucosidic residue at C4 was the observation of the formation of furosine. Furosine is formed by the reaction of the ϵ-amino group of lysine with saccharides and possesses an unsubstituted furan ring (Figure 5).

Therefore, furosine should be the reaction product of an elimination reaction at C4. When the formation of furosine was compared in the reaction of glucose and maltose with Boc-lysine at 120 °C in a quasi-water-free system, there was a clear preference for furosine formation in the mixtures containing maltose. At a comparable degree of browning (E_{420} = 4.12), which was reached by maltose/Boc-lysine under the chosen condi-

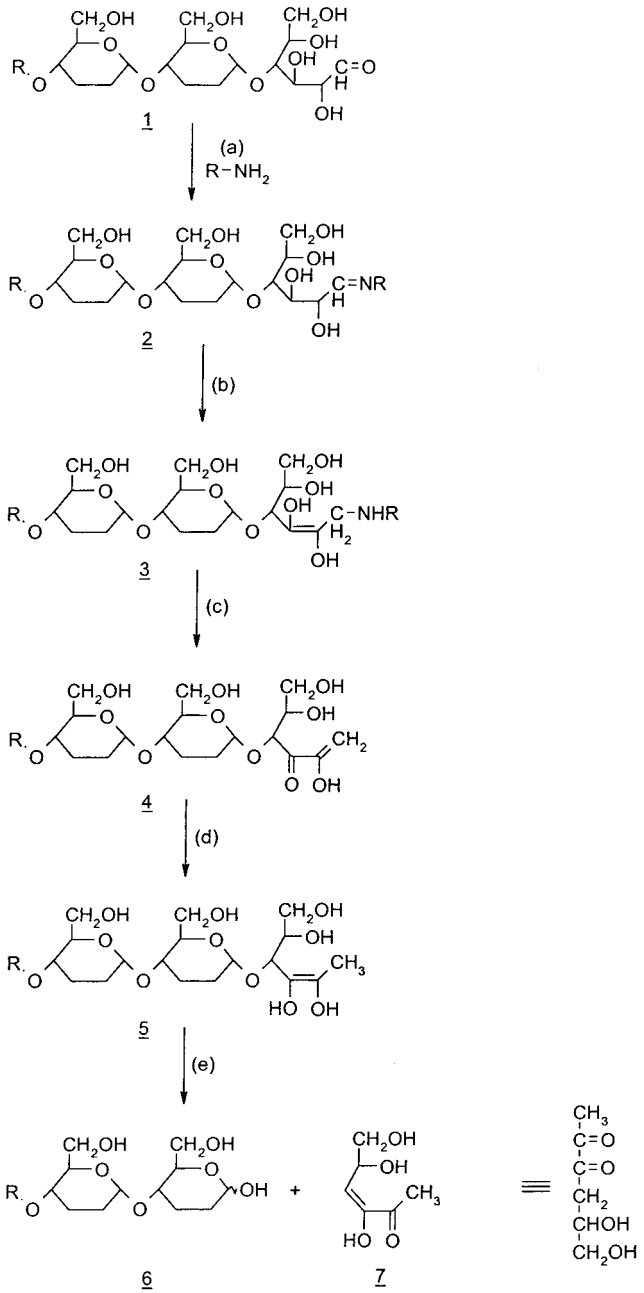

Figure 4. Proposed peeling off mechanism for the formation of 1,4-DDH from oligosaccharide.

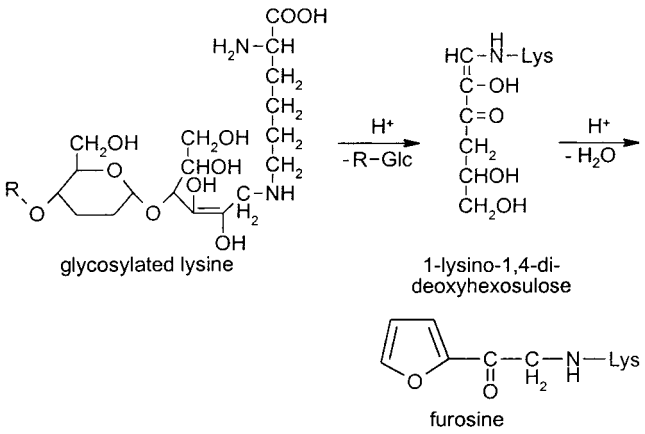

Figure 5. Formation of furosine from glycosylated lysine (R = H for maltose or R = Glc for maltotriose).

Table 2. Degradation of Maltose and Formation of Maltulose, mol %, in Caramelization (cara; Mal/OPD) and the Maillard Reaction (MR; Mal/Gly/OPD) and Maltulosylglycine in Mal/Gly/OPD[a]

time, min	maltose		maltulose		maltulosylglycine
	cara	MR	cara	MR	MR
0	100	100	0	0	0
30	16.58	15.50	0.09	nd	3.27
60	13.85	15.90	0.13	nd	6.80
120	10.39	15.17	0.20	0.72	10.63
180	13.02	8.14	0.35	0.65	10.78
240	10.47	3.41	0.44	0.41	8.26

[a] nd = not detected.

tions after 15 min, a furosine concentration of 6 mmol/L was detected, whereas Glc/Boc-lysine gave an absorbance of $E_{420} = 3.95$ after 30 min, but only 0.2 mmol/L furosine was produced. This pronounced difference of more than a degree of magnitude is logical when one considers that the precursor of furosine, the Amadori rearrangement product, has, in the case of maltose, only a limited number of degradation pathways. The formation of the 1-lysino-1,4-dideoxyosone should be favored in order to eliminate the rather bulky glucosidic residue. Cyclization and dehydration of 1-lysino-1,4-dideoxyosone easily gives rise to furosine. In the case of glucose the elimination of water and subsequent formation of an α-dicarbonyl should, from the steric point of view, occur more or less equally at C1, C3, or C4. Since the reaction mixture is treated with very strong acid, even the elimination of water at C3 should be favored, forming 3-DH. There is no reason elimination at C4 and subsequent formation of furosine should play a comparable role in glucose degradation as in the case of maltose.

Degradation of Maltose Formation of Glucose. Maltose degradation is slower than that of Glc, both in caramelization and in the MR. However, the majority was readily converted in the first 60 min, but after 240 min there still remained a small concentration unreacted. The difference in degradation of the starting compound between caramelization and the MR was rather small (Table 2).

The identity of glucose was additionally confirmed by HPLC/PAD. In caramelization not much more glucose was detected than was originally present in maltose as an impurity; apparently breakdown of the disaccharide into monosaccharides is not important in quasi-water-free caramelization (Figure 6). The earlier postulated formation of a glycosyl cation might in this case preferentially lead to the formation of anhydrosugars and transglycosylation products (Häseler and Kroh, 1998).

In the MR, however, glucose reached with 4.4 mol % a relatively high concentration at 60 min and was degraded to 1 mol % at 240 min. It is interesting to see that the maximum concentration of glucose was observed at a point where the 1,4-DDH concentration began to increase strongly (Table 1); therefore, the formation of glucose in the case of the MR can be seen as additional evidence for the proposed peeling off mechanism, yielding glucose as the second reaction product when starting from maltose.

Isomerization to Maltulose. In all systems containing maltose/OPD with or without glycine, formation of

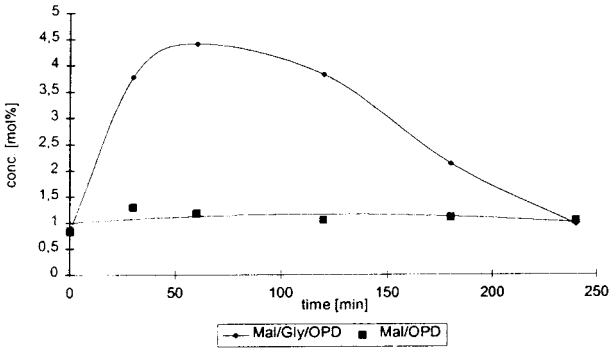

Figure 6. Formation of glucose from maltose in caramelization (Mal/OPD) and in the Maillard reaction (Mal/Gly/OPD).

maltulose was observed. However, the concentration of maltulose was rather small. There was no significant difference in the extent of isomerization observable; only the course of the reaction appeared to differ between caramelization and the MR (Table 2).

In the latter system, maltulose was detected only after 120 min and was degraded again readily, whereas in caramelization a small but steady increase of maltulose was seen. The observation that the maltulose concentration was rising in caramelization throughout the observed time range can be explained by the limited number of other pathways for maltulose degradation compared to the Maillard reaction. Moreover, more starting compound, maltose, was available throughout caramelization than in the MR. However, due to a maximum concentration of maltulose of less than 1 mol % in both systems, this reaction product was almost negligible.

Formation of Maltulosylglycine. Maltulosylglycine was formed somewhat slower than the Amadori rearrangement product from glucose; at 60 min when most of the maltose had already been converted, the concentration of maltulosylglycine was still rising (Table 2). The highest concentration was formed at about 150 min with ≈11 mol %; the maximum was not as sharp as in the case of Glc/Gly/OPD, indicating that neither production nor degradation of the product was as fast as in the case of the monosaccharide. After 240 min the major part of the Amadori rearrangement product (8.3 mol %) had not degraded.

Summarizing the obtained results, it is evident that disaccharides show a different behavior in the MR than monosaccharides.

Most distinct is the enhanced formation of 1,4-DDH from disaccharides, by the proposed peeling off mechanism, which is most probably caused by the glucosyl substituent. Additionally it was shown that maltose is degraded slower and that also the Amadori rearrangement product is formed slower than in the case of the monosaccharide.

Reactions of Trisaccharides. *Formation of α-Dicarbonyls.* To confirm the validity of the hypothesis for the degradation of oligosaccharides with a higher degree of polymerization, the same set of experiments was carried out with maltotriose. Looking at the formation of α-dicarbonyls from the trisaccharide, the pathway postulated for maltose seems to be repeated.

Production of α-dicarbonyls occurred even slower than in reaction mixtures with maltose, and therefore samples were taken over a time range of 8 h. As for maltose in caramelization as well as in the MR, 1,4-DDH was again

Table 3. Formation of α-Dicarbonyls Detected as Quinoxalines, mol %, in Caramelization (Maltotriose/OPD) and in the MR (Maltotriose/Gly/OPD)[a]

time,	1-DH		3-DH		1,4-DDH		glyoxal		methylglyoxal	
min	cara	MR	cara	MR	cara	MR	cara	MR	cara	MR
30	0.03	0.03	nd	0.00	0.06	0.09	0.07	0.13	0.15	0.32
60	0.07	0.08	nd	0.04	0.19	0.47	0.17	0.49	0.47	1.84
120	0.07	0.27	nd	0.08	0.65	1.57	0.47	1.05	0.96	3.44
180	0.10	0.46	nd	0.12	1.16	3.15	0.62	1.12	1.69	3.87
240	0.14	0.56	nd	0.21	1.57	4.02	0.91	nr	2.34	3.26
360	0.23	1.57	nd	0.35	2.80	9.76	1.47	nr	2.92	3.49
480	0.27	1.58	nd	0.37	3.91	11.72	1.67	nr	2.89	3.24

[a] nr = not resolved, nd = not detected.

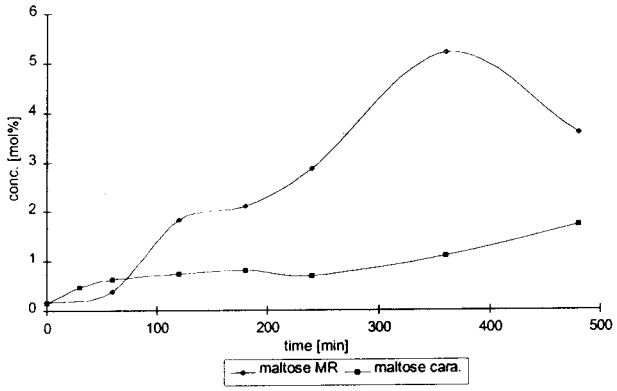

Figure 7. Formation of maltose from maltotriose in caramelization (maltotriose/OPD) and in the MR (maltotriose/Gly/OPD).

by far the most abundant α-dicarbonyl detected, showing clearly the differences from glucose as a monosaccharide.

In caramelization the maximum concentration of 1,4-DDH was nearly 4 mol % after 480 min. Further only sugar fragments possessing α-dicarbonyl structure could be quantified at that time in considerable amounts, such as methylglyoxal with almost 3 mol % and glyoxal with more than 1.5 mol % (Table 3).

In the presence of glycine a phenomenon similar to that for maltose was observed. Formation of 1,4-DDH was increased strongly, and even after 8 h, when nearly 12 mol % 1,4-DDH was formed, the final concentration had not been reached. This could be due to various reasons. First, maltotriose was degraded slower than the aforementioned saccharides, lending itself longer to the peeling off mechanism. Second, the second reaction product of a peeling off reaction besides 1,4-DDH would be maltose, which in turn could react again in terms of a second peeling off step. Indeed the formation of maltose was observed in MR mixtures; the strong increase of 1,4-DDH between 240 and 360 min occurred parallel to enhanced production of maltose. This finding can be seen as further evidence for the proposed peeling off mechanism (Figure 7).

Formation of other α-dicarbonyl compounds was not increased as much. Methylglyoxal was now formed earlier, but remained at a concentration of slightly more than 3 mol %, and also 1-DH was detected to some extent but at a maximum of 1.6 mol % only played a minor role compared to 1,4-DDH.

Degradation of Maltotriose. Just as for glucose and for maltose, it was found that the major part of the starting compound was degraded in the first hour. Nevertheless, maltotriose remained longer in caramel-

Table 4. Degradation of Maltotriose, mol %, in Caramelization (Maltotriose/OPD) and the Maillard Reaction (Maltotriose/Gly/OPD)

time, min	0	30	60	120	180	240	360	480
maltotriose/OPD	100	36.31	30.74	28.28	32.35	31.35	29.65	25.82
maltotriose/Gly/OPD	100	25.06	20.77	20.16	18.24	16.44	17.38	9.80

ization and in MR mixtures than the other sugars. As published elsewhere this is well in line with the finding that saccharides become less reactive with increasing degree of polymerization.

In the case of caramelization after 8 h there was still more than 25 mol % present. In the MR at that time only 9.8 mol % was present (Table 4). The addition of equimolar amounts of glycine increased the degradation markedly.

Degradation of maltotriose in caramelization differs from that in the Maillard reaction, which is underlined additionally by the fact that in caramelization carbohydrates larger than maltotriose were detected on the HPTLC plate in this system. They are probably formed by transglycosylation.

Formation of Maltose and Maltotriulose. For the formation of maltose a similar observation was made as for glucose formation from maltose. In both caramelization and the MR maltose was formed. In caramelization a slow but steady increase of the maltose concentration was observable. During the long reaction time, splitting off of a glycosyl cation from the nonreducing end of the molecule would lead to the formation of maltose from maltotriose. After 8 h maltose was still being formed, which is consistent with the finding that there was still a considerable amount of maltotriose present (Figure 7).

In the MR the concentration of maltose rose to 5.2 mol % at 360 min and then decreased again to 3.6 mol % after 8 h. Several pathways could lead to its formation: besides by formation of a glucosyl cation in MR, maltose might also be formed by the peeling off reaction that occurs at a higher rate in the later phase of the reaction. The decrease of maltose in the advanced stage of the reaction can be explained by the slower formation of maltose due to the decreasing concentration of maltotriose, but also by the fact that maltose could readily react again with glycine, in terms of a second peeling off step, competing with the less reactive maltotriose.

Maltotriulose could only be assigned tentatively by color and R_f value on the HPTLC plate, since there was no reference material available. Comparing the absolute values on one plate, in the cases of both the MR and caramelization, the concentration of maltotriulose remained at the initial value.

The Amadori rearrangement product could also only be assigned by R_f value and color of the spot. Roughly, one can say that the highest concentration of the Amadori rearrangement product was reached after 30 min, and at 480 min maltotriulosylglycine could no longer be detected. No maltulosylglycine or fructosylglycine could be detected on the HPTLC plate.

CONCLUSIONS

The reactivity of the di- and trisaccharides under quasi-water-free conditions decreased in comparison to that of glucose due to the increasing degree of polymerization. However, between the oligosaccharides no qualitative difference was observed; when the reaction time was prolonged, maltotriose had reacted to a degree comparable to that of maltose after a shorter reaction time. Degradation of the starting compound led to α-dicarbonyls, isomerization products, smaller carbohydrates, and, in the case of the reaction with glycine, the Amadori rearrangement product as important intermediates in the Maillard reaction.

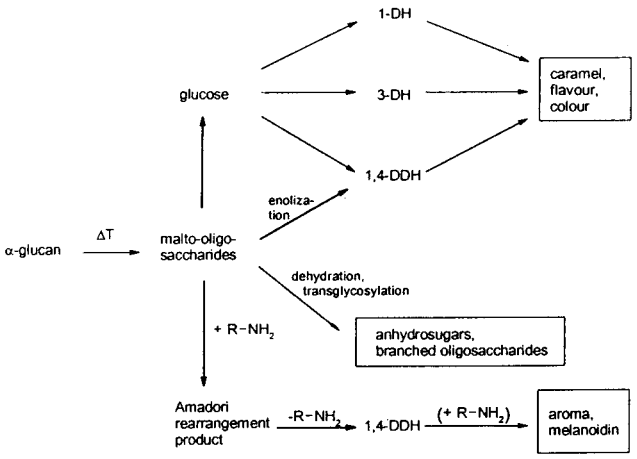

Figure 8. Schematic reactions of *malto*-oligosaccharides in caramelization and the Maillard reaction.

Formation of α-dicarbonyls most probably occurs by reaction of the free glycosidic OH group, catalyzed by an amino compound, with subsequent enolization and elimination of water or by retro-aldolization. In contrast to monosaccharides oligosaccharides generated 1,4-DDH as the predominating α-dicarbonyl. Most probably this pathway is favored due to the glycosidic substituent at C4, which might block other reaction mechanisms. The reaction via vinylogous β-elimination of the glycosidic residue at C4 and subsequent formation of the 1,4-DDH was strongly preferred. Elimination at C4 appears to be the only pathway that leads to degradation of the oligosaccharide from the reducing end.

Under MR conditions, formation of α-dicarbonyls from *malto*-oligosaccharides is more or less limited to formation or 1,4-DDH (Figure 8). Other reactions, such as dehydration and transglycosylation, lead to rather unreactive intermediates (Kroh et al., 1996) and therefore may not be as relevant for the further reaction of *malto*-oligosaccharides.

1,4-DDH is a reactive intermediate and a precursor of various heterocyclic volatile compounds. Due to its α-dicarbonyl moiety, it is likely to take part in condensation reactions, and therefore might play a role in formation of melanoidin.

Finally the α-dicarbonyls, and in the case of oligosaccharides especially 1,4-DDH, influence the production of flavor and color a great deal.

ACKNOWLEDGMENT

We express our sincere gratitude to Mrs. Anette Berghäuser for excellent technical assistance. We are also indebted to Dr. Clemens Mügge, Humboldt University Berlin, for NMR measurements. Further gratitude is due to Dr. Martinus I. J. S. van Boekel and

Carline Brands, Wageningen Agricultural University, for determination of furosine.

LITERATURE CITED

Alltech Nederland, Breda. Determination and quantification of Furosine in heat treated dairy products by means of HPLC, extracted from *Gazetta Ufficiale della Republica Italiana (24-3-94)*.

Beck, J.; Ledl, F.; Severin, T. Formation of 1-deoxy-D-erythro-2,3-hexodiulose from Amadori compounds. *Carbohydr. Res.* **1988**, *177*, 240−243.

Glomb, M. A.; Pfahler; C. Synthese eines bedeutenden Maillard-Intermediats beim Abbau von Hexosen. *Lebensmittelchemie* **1999**, *53*, 16−17.

Häseler, J.; Kroh, L. W. Nonvolatile reaction products of heat-induces degradation of α-glucans; Part II: Analysis of short-chain α-glucans by gas chromatography/mass spectrometry. *Z. Lebensm.-Unters.-Forsch. A* **1998**, *207*, 44−49.

Hayashi, T.; Mase, S.; Namiki, M. Formation of Three-carbon Sugar Fragment at an Early Stage of the Browning Reaction of Sugar with Amines or Amino Acids. *Agric. Biol. Chem.* **1986**, *50*, 1959−1964.

Hodge, J. E.; Fisher, B. E. Amadori Rearrangement Products. In *Methods in Carbohydrate Chemistry II*; Whistler, W., Ed.; Academic Press Inc.: New York, 1963.

Hodge, J. E. Origin of Flavour in Food. Nonenzymatic browning reactions. *Chemistry and Physiology of Flavours*; AVI: Wersport, CT, 1967.

Hofmann, T.; Bors, W.; Stettmaier, K. Studies on Radical Intermediates in the Early Stage of the Nonenzymatic Browning of Carbohydrates and Amino Acids. *J. Agric. Food Chem.* **1999**, *47*, 379−390.

Hollnagel, A.; Kroh, L. W. Formation of α-dicarbonyl fragments from mono- and disaccharides under caramelisation and Maillard reaction conditions. *Z. Lebensm.-Unters.-Forsch. A* **1998**, *207*, 50−54.

Kato, Y.; Matsuda, T.; Kato, N.; Nakamura. Maillard Reaction of Disaccharides with Protein: Suppressive Effect of Non-reducing End Pyranoside Groups on Browning and Protein. *J. Agric. Food Chem.* **1989**, *37*, 1077−1081.

Kroh, L. W.; Jalyschko, W.; Häseler, J. Non-volatile Reaction Products by Heat-induced Degradation of α-Glucans. Part I: Analysis of oligomeric Maltodextrins and Anhydrosugars. *Starch/Stärke* **1996**, *48*, 426−433.

Kroh, L. W.; Schröder, R.; Mügge, C.; Westphal, G.; Baltes, W. Thermisch induzierter Abbau von Disaccharid-Amadori-Verbindungen in quasi wasserfreiem Reaktionsmilieu. *Z. Lebensm.-Unter.-Forsch.* **1992**, *194*, 216−221.

Ledl, F.; Schleicher, E. Die Maillard-Reaktion in Lebensmitteln und im menschlichen Körper- neue Ergebnisse zu Chemie, Biochemie und Medizin. *Angew. Chem.* **1990**, *102*, 597−734.

Madson, M. A.; Feather, M. S. An improved preparation of 3-deoxy-D-erythro-hexos-2-ulose via Bis-(benzoyl)hydrazone and some related constitutional studies. *Carbohydr. Res.* **1981**, *94*, 183−191.

Morita, N.; Hayashi, K.; Takagi, M.; Miyano, K. Gas-Liquid Chromatography and Mass Spectrometry of Quinoxalines Derived from Various Homoglucans by Alkaline o-Phenylenediamine Method. *Agric. Biol. Chem.* **1983**, *47*, 757−763.

Morita, N.; Inoue, K.; Takagi M. Quinoxalines Derived from D-Glucose and *o*-Phenylenediamine in a Weakly Acidic Medium. *Agric. Biol. Chem.* **1981**, *45*, 2665−2668.

Nedvidek, W.; Ledl, F.; Fischer, P. Detection of 5-hydroxymethyl-2-methyl-3(2H)-furanone and of α-dicarbonyl compounds in reaction mixtures of hexoses and pentoses with different amines. *Z. Lebensm.-Unters.-Forsch.* **1992**, *194*, 222−228.

Pischetsrieder, M.; Severin, T. Maillard Reaction of Maltose-Isolation of 4-(Glucopyranosyloxy)-5-(hydroxymethyl)-2-3(2H)-furane. *J. Agric. Food Chem.* **1994**, *42*, 890−892.

Pischetsrieder, M.; Schoetter, C.; Severin, T. Formation of an Aminoreductone during the Maillard Reaction of Lactose with N^{α}-Acetyllysine or Proteins. *J. Agric. Food Chem.* **1998**, *46*, 928−931.

Wedzicha, B. L.; Kedward C. Kinetics of the oligosaccharides-glycine-sulphite reaction: relationship to the browning of oligosaccharide mixtures. *Food Chem.* **1995**, *54*, 397−402.

Weenen, H. Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chem.* **1998**, *62*, 393−401.

Received for review June 8, 1999. Accepted July 28, 2000. This study was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific program, FAIR-CT96-1080, *Optimization of the Maillard reaction. A way to improve quality and safety of thermally processed foods*.

JF9906127