Eur Food Res Technol (1999) 209:113–121

ORIGINAL PAPER

T. Hofmann

Quantitative studies on the role of browning precursors in the Maillard reaction of pentoses and hexoses with L-alanine

Received: 15 October 1998

Abstract The time course of the formation of 1- and 3-deoxyosones, 2-osuloses, furan-2-aldehydes, as well as C_2, C_3 and C_4 α-dicarbonyl compounds, and hydroxycarbonyl compounds, upon the thermal treatment of aqueous solutions of glucose or xylose in the presence of L-alanine was investigated. 2-Osuloses and glyoxal were formed independently of the carbohydrate moiety, predominantly at the very beginning of the heating process, whereas the formation of 1- and 3-deoxyosones, 2-oxopropanal and hydroxy-2-propanone was favoured with increasing reaction times. In order to evaluate the role of these carbohydrate degradation products in the development of browning their activities as browning precursors were determined on the basis of a dosage/activity relationship by combining chemical/instrumental techniques and visual/sensory analysis. The browning precursors 3-deoxyosone, glyoxal and glycolaldehyde showed highest activities at the very beginning of heating carbohydrate/L-alanine solutions. In contrast to glucose, 2-oxopropanal was found to be an effective colour precursor even at the beginning of heating xylose in the presence of L-alanine. A prolongation of the reaction time led to a drastic decrease in the precursor activity of glyoxal, independently of that of the carbohydrate moiety, and, in parallel, to an increase in the participation of 2-oxopropanal and also furan-2-carboxaldehyde in browning reactions with L-alanine. In the present study, certain carbohydrate degradation products could be identified as browning precursors, and it could be demonstrated for the first time that the activity of these reaction intermediates in producing browning substances changes during the reaction time.

T. Hofmann
Deutsche Forschungsanstalt für Lebensmittelchemie,
Lichtenbergstrasse 4, D-85748 Garching, Germany
e-mail: hofmann@dfa.leb.chemie.tu-muenchen.de

Key words Carbohydrate fragmentation · Glyoxal · Glycolaldehyde · Maillard reaction

Introduction

The Maillard reaction between reducing carbohydrates and amino acids is chiefly responsible for the development of the desirable browning of foods, e.g. during thermal processing of foods, such as roasting of meat or coffee, baking of bread or kiln-drying of malt, and also for undesirable colour formation, e.g. during the preparation of condensed milk or drying of fruit products. To further improve the quality of foods by controlling the non-enzymatic browning reaction more efficiently, a better understanding of the colourless browning precursors, originating from carbohydrate degradation, is required.

As outlined in Fig. 1, in the reaction of carbohydrates (I) with amino acids, glycosylamines (II) are formed as the first reaction products, which are then easily transformed into deoxyglycosyl amino acids (III) via the so-called Amadori rearrangement [1]. These intermediates are not stable and are easily further degraded by loss of the amino acid to form 1- and 3-deoxyosones (IVa and IVb). Besides these deoxyosones, 2-osuloses can also be formed from an enaminol intermediate of the Amadori rearrangement upon transition-metal-catalyzed oxidation by oxygen in the air [2–4]. Due to their reactive functional groups, the α-dicarbonyl compounds deoxyosones and 2-osuloses are very reactive, and can either be further degraded by cyclization resulting in products with intact carbon chains, or by fragmentation reactions leading to C_2, C_3 and C_4 carbonyl compounds. These so-called advanced Maillard reaction products are discussed as important intermediates in browning [5].

Hayashi and Namiki [6] reported that the carbohydrate cleavage products, glycolaldehyde and 2-oxopropanal, showed extremely high reaction rates when

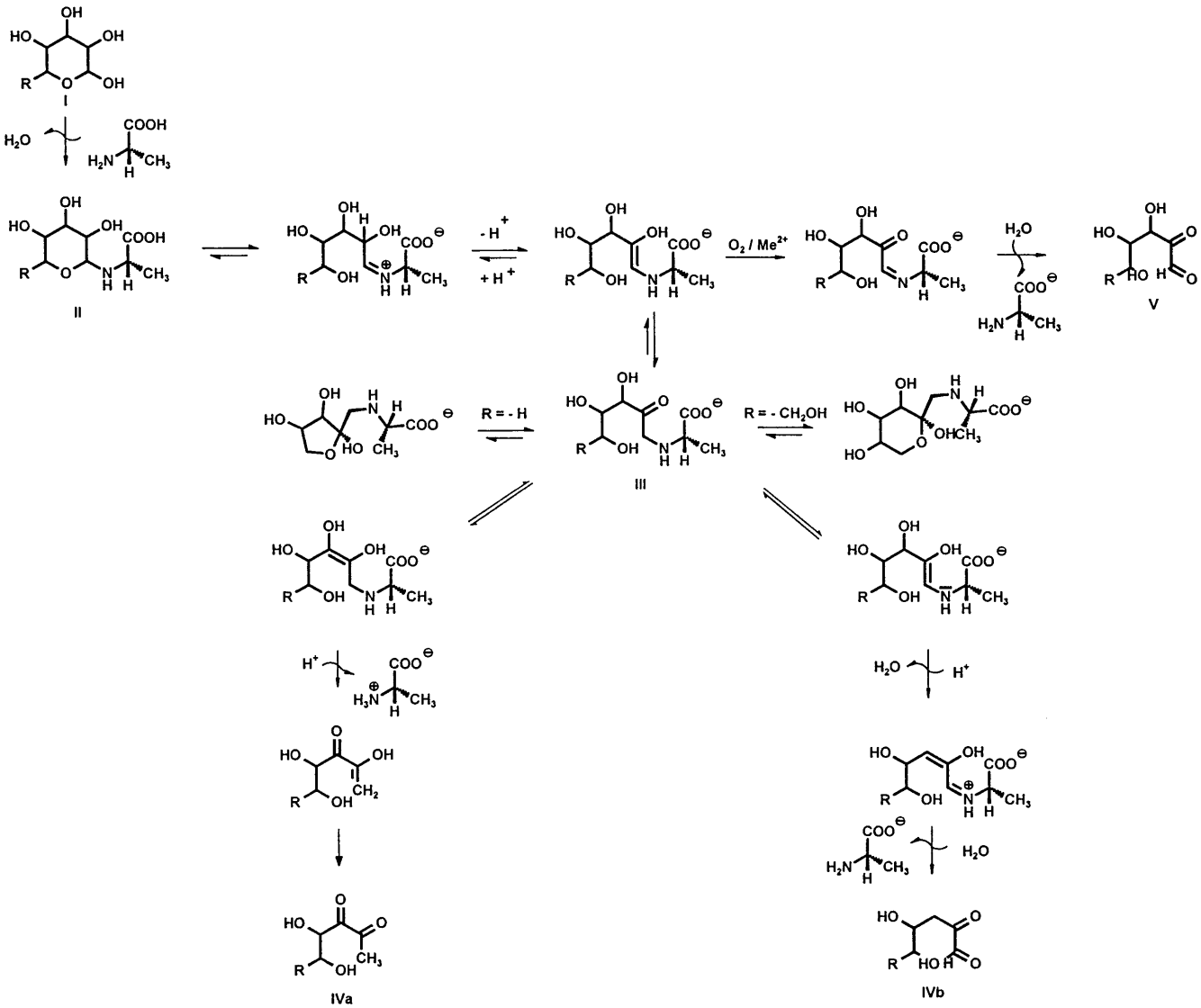

Fig. 1 Formation of 2-osuloses, 1-deoxy-2,3-diuloses and 3-deoxy-2-osuloses from carbohydrates and L-alanine

browning with β-alanine, compared to reducing carbohydrates, and even to other reactive carbohydrate degradation products. In contrast to the results of these experiments performed with equimolar reaction mixtures of β-alanine and carbonyl compounds, the carbohydrate degradation products formed during the thermal treatment of Maillard mixtures are produced in non-equimolar ratios, and usually significantly differ in their yields. The role of glycolaldehyde and 2-oxopropanal as precursors in non-enzymatic browning is, therefore, as yet very unclear.

Several quantitative studies have been, therefore, performed to determine the amounts of dicarbonyl products from carbohydrate degradation, e.g. 1- and 3-deoxyosones, glyoxal, 2-oxopropanal or butane-2,3-dione [7–13]. In most studies, carbohydrate/amino acid mixtures were heated in the presence of surplus amounts of 1,2-diaminobenzene in order to transform these reactive α-dicarbonyl intermediates in situ into their stable quinoxaline derivatives, which were then analysed by HPLC or GC [7–10, 13]. This in situ trapping technique, however, leads to an accumulation of the derivatized target compounds during heating of the Maillard mixtures and, consequently offers no insight into the changes of the actual amounts of these reaction intermediates produced at certain stages of the carbohydrate/amino acid reaction.

Very recently, Maillard mixtures were, therefore, heated without a trapping reagent and, after cooling, the dicarbonyls formed were derivatized with 1,2-diaminobenzene prior to quantification [11]. Because these experiments were, however, performed in butanol/water solutions in alkaline media, which are not used in food processing, the results obtained offered no insight into the carbohydrate fragmentation which occurs during the cooking of foods.

Besides dicarbonyl compounds, C_2, C_3 and C_4 hydroxycarbonyl compounds, such as glycolaldehyde, hy-

droxy-2-propanone or 3-hydroxy-2-butanone, are also known to be liberated upon heating carbohydrates in the presence of amino acids. However, no analytical techniques are as yet available to exactly quantify these hydroxycarbonyls. Consequently, the role of these reaction intermediates as browning precursors remain unclear.

The purpose of the present investigation was, therefore, to determine the time course of the formation of dicarbonyl and hydroxycarbonyl intermediates formed from xylose or glucose, when heated in the presence of L-alanine under cooking conditions, and, in addition, to evaluate the role of these carbohydrate degradation products as browning precursors in the formation of colour on the basis of dosage/activity relationships.

Materials and methods

Chemicals. The following compounds were obtained commercially: glucose, xylose, L-alanine (Fluka, Neu-Ulm, Germany); glycolaldehyde, hydroxy-2-propanone, 2-hydroxy-3-butanone, glyoxal, 2-oxopropanal, butane-2,3-dione, furan-2-carboxaldehyde, 5-(hydroxymethyl)furan-2-carboxaldehyde, quinoxaline, 2-methylquinoxaline, 2,3-dimethylquinoxaline, ethoxamine hydrochloride and 1,2-diaminobenzene (Aldrich, Steinheim, Germany). 1,2-Diaminobenzene was recrystallized twice from methanol, and furan-2-carboxaldehyde was freshly distilled in vacuo prior to use.

2-Methyl-3-(1,2,3-trihydroxypropyl)quinoxaline, 2-(2,3,4-trihydroxybutyl) quinoxaline, 2-methyl-3-(1,2-dihydroxyethyl)quinoxaline and 2-(2,3-dihydroxy-propyl) quinoxaline was prepared as recently reported [4, 14]. 2-(1,2,3-Trihydroxypropyl)quinoxaline and 2-(1,2,3,4-tetrahydroxybutyl)quinoxaline were synthesized by reacting 2-xylosulose and 2-glucosulose [15], respectively, with 1,2-diaminobenzene following the procedure described recently for 2-(2,3,4-trihydroxybutyl)quinoxaline [4]. The ^{13}C-labelled standards [$^{13}C_4$]-butane-2,3-dione and [$^{13}C_4$]-3-hydroxy-2-butanone were synthetised by closely following procedures reported in the literature [16].

Maillard reaction mixtures. Solutions of 6 mmol xylose or 6 mmol glucose in 4 ml phosphate buffer (0.5 mol/l, pH 7.0) were refluxed in the presence of 6 mmol L-alanine for the reaction times given in Figs. 3–6.

Quantification of 2-pentosulose, 1-deoxy-2,3-pentodiulose, 3-deoxy-2-pentosulose, 2-hexosulose, 1-deoxy-2,3-hexodiulose and 3-deoxy-2-hexosulose. A quarter of the reaction mixture was withdrawn, rapidly cooled to room temperature, and 1 ml water and 2 ml of a methanolic solution of 1,2-diaminobenzene (1 mol/l) were added; the mixtures were maintained for 3 h at 30 °C. The mixtures were diluted with water and then analysed by RP-HPLC using a solvent gradient starting with a mixture (10/90; v/v) of acetonitrile and ammonium formate buffer (pH 3.5; 20 mmol/l) and increasing the acetonitrile content to 30% within 50 min. By monitoring the effluent at a wavelength of 320 nm, 2-hexosulose, 1-deoxy-2,3-hexodiulose, 3-deoxy-2-hexosulose, 2-pentosulose, 1-deoxy-2,3-pentodiulose and 3-deoxy-2-pentosulose were detected as their corresponding quinoxaline derivatives at the retention times (R_t) given in parentheses: 2-(1,2,3,4-tetrabutyl)quinoxaline (R_t 14.2 min), 2-methyl-3-(1,2,3-trihydroxypropyl) quinoxaline (R_t 15.8 min), 2-(2,3,4-trihydroxybutyl)quinoxaline (R_t 17.2 min), 2-(1,2,3-trihydroxypropyl)quinoxaline (R_t 20.7 min), 2-methyl-3-(1,2-dihydroxyethyl)quinoxaline (R_t 22.2 min) and 2-(2,3-dihydroxypropyl)quinoxaline (R_t 23.4 min). Quantification of these quinoxalines was performed by comparing the peak areas obtained at 320 nm with those of standard solutions containing known amounts of each pure, synthetic, reference compound.

Quantification of glycolaldehyde, hydroxy-2-propanone and 2-hydroxy-3-butanone by stable isotope dilution analysis. The reacted Maillard mixtures were rapidly cooled to room temperature, the internal standard, [$^{13}C_4$]-3-hydroxy-2-butanone, dissolved in methanol, and, after equilibration, 4 mmol ethoxamine hydrochloride was added. The pH was adjusted to 7.5 with aqueous sodium hydroxide (0.1 mol/l) and the mixtures were maintained for 3 h at 30 °C. The pH was then adjusted to 6.0 with hydrochloric acid (0.1 mol/l) and the mixtures were extracted with three volumes of 15 ml diethyl ether. After drying over sodium sulphate the organic layer was concentrated to about 3 ml and applied to a GC/MS system. For the determination of the response factors (R_f), which are given in parentheses, solutions containing defined amounts of glycolaldehyde (R_f 0.96), hydroxy-2-propanone (R_f 0.99) or 2-hydroxy-3-butanone (R_f 1.00), mixed with [$^{13}C_4$]-3-hydroxy-2-butanone in ratios of 3:1 to 1:3, were treated as described above for the Maillard mixtures. Quantification was performed by MS using a GC coupled to a MS which scanned the pseudomolecular ions $[M+1]^+$ of the syn- and anti-O-ethyloxime obtained in the chemical ionization (CI) mode. The following results were obtained GC/MS-CI of syn/anti-*O*-ethyl glycolaldehyde oxime (R_t 5.73/6.54 min), 104 (100, $[M+1]^+$); GC/MS-CI of syn/anti-*O*-ethyl hydroxy-2-propanone oxime (R_t 7.45/8.15 min) 118 (100, $[M+1]^+$); GC/MS-CI of syn/anti-*O*-ethyl 2-hydroxy-3-butanone oxime (R_t 8.34/9.00 min), 132 (100, $[M+1]^+$); GC/MS-CI of syn/anti-*O*-ethyl [$^{13}C_4$]-3-hydroxy-2-butanone oxime (R_t = 8.34/9.00 min), 136 (100, $[M+1]^+$).

Quantification of glyoxal, 2-oxopropanal and butan-2,3-dione by stable isotope dilution analysis. A quarter of the reaction mixture was withdrawn, rapidly cooled to room temperature and diluted with 5 ml water. The solution was adjusted to pH 6.5 with hydrochloric acid (0.1 mol/l), the internal standard, [$^{13}C_4$]-butane-2,3-dione, was dissolved in methanol, and 2 ml of a methanolic solution of 1,2-diaminobenzene (1 mmol/l) was added. After maintaining the mixture for 3 h at 30 °C, the pH was adjusted to 5.0 with hydrochloric acid (0.1 mol/l) and the mixtures were extracted with 3 volumes of 15 ml diethyl ether. After drying over sodium sulphate the organic layer was concentrated to about 0.5 ml and the liquid phase was applied to the top of a column (5 × 50 mm) filled with a slurry of silica gel (1 g) in *n*-pentane. Flushing the column with 15 ml of a pentane/diethyl ether mixture (30/70, v/v) under a stream of nitrogen yielded an eluate; this was concentrated to about 2 ml and analysed by GC/MS. For the determination of the R_f, which are given in parentheses, solutions containing known amounts of glyoxal (R_f 0.97), 2-oxopropanal (R_f 0.97) and butane-2,3-dione (R_f 1.00), mixed with [$^{13}C_4$]-butane-2,3-dione in ratios of 1:3–3:1, were treated as discribed above for the Maillard mixtures. Quantification was performed by MS using a GC coupled to a MS which scanned the molecular ions of the quinoxaline derivatives obtained in the CI mode. The following results were obtained: GC/MS-CI of quinoxaline (R_t 13.09 min): 131 (100, $[M+1]^+$); GC/MS-CI of 2-methylquinoxaline (R_t 15.35 min), 145 (100, $[M+1]^+$); GC/MS-CI of 2,3-dimethylquinoxaline (R_t 17.73 min), 159 (100, $[M+1]^+$); GC/MS-CI of [$^{13}C_4$]-2,3-dimethylchinoxaline (R_t 17.73 min), 163 (100, $[M+1]^+$).

Quantification of furan-2-carboxaldehyde and 5-(hydroxymethyl-)furan-2-carboxaldehyde. After cooling the reaction mixtures, specific amounts of thiophen-2-carboxaldehyde were added to the reaction mixtures as the internal standard. After extraction of the analytes and the standard with four volumes of 60 ml diethyl ether, followed by drying over sodium sulphate, quantification of furan-2-carboxaldehyde and 5-(hydroxymethyl)furan-2-carboxaldehyde was performed by MS. MS response factors of 0.97 and 0.94 were calculated from mixtures containing known amounts of furan-2-carboxaldehyde, 5-(hydroxymethyl)-furan-2-carboxaldehyde and thiophen-2-aldehyde by using the pseudomolecular ions

(m/z 97, m/z 127 and m/z 113, respectively) obtained in the CI mode.

Determination of the colour intensity of browned carbohydrate/L-alanine solutions. For the determination of the browing intensity the complete reaction mixtures were diluted step by step (1+1, by volume) until a colour difference between the sample (5 ml) and two blanks (tap water; 5 ml) in a glass vial (1 cm i.d.) could just be detected visually using a triangle test. Using this procedure, a colour dilution (CD) factor could be defined for each reaction mixture [17].

High performance liquid chromatography. The HPLC apparatus (Kontron, Eching, Germany) consisted of two pumps (type 422), a gradient mixer (M 800) and a Rheodyne injector (100-μl loop). The effluent was monitored by a diode array detector (type 440, Kontron) operating in a wavelength range between 220 nm and 500 nm. Separations were performed on a stainless steel column (250×4.6 mm; Shandon, Frankfurt, Germany) packed with RP-18 material (5 μm Hypersil Shandon) with a flow rate of 0.6 ml/min.

High resolution gas chromatography/mass spectrometry. HRGC was performed with a type 5160 GC (Fisons, Mainz, Germany) by using a capillary SE-54 column (30 m×0.32 mm fused silica capillary column, DB-5, 0.25 μm; J&W Scientific, Fisons, Mainz, Germany). The samples were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at a rate of 40 °C/min to 50 °C then held for 2 min, then raised at a rate of 6 °C/min to 230 °C, and held for 5 min. The flow of the carrier gas, helium, was 2.5 ml/min. MS analysis was performed with an MS 95 S (Finnigan, Bremen, Germany) in tandem with the HRGC. MS in the CI mode (MS/CI) was performed at 115 eV with isobutane as the reactant gas.

Results

To gain an insight into the role of carbohydrate degradation products in the formation of non-enzymatic browning substances, we, at first, were primarily interested in the actual amounts of 1- and 3-deoxyosones, as well as 2-osuloses, formed from carbohydrates in the presence of L-alanine, i.e. those dicarbonyl compounds with the original carbon backbone. Because 2-osuloses as well as 1- and 3-deoxyosones exist in aqueous solution as a complex mixture of various hemiacetal and hemiketal forms [5], these cyclic forms were, therefore, converted into one quinoxaline derivative by reaction with 1,2-diaminobenzene, as shown for the 3-deoxy-2-hexosulose in Fig. 2.

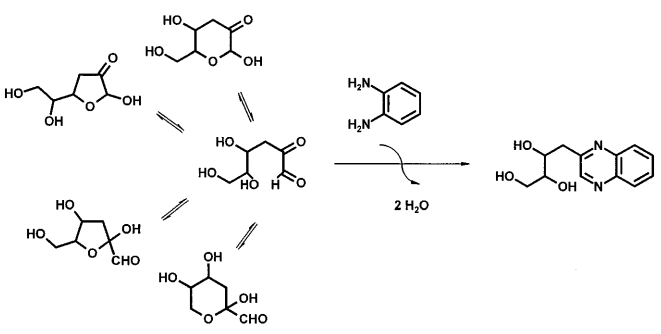

Fig. 2 Derivatization of 3-deoxy-2-hexosulose with 1,2-diaminobenzene

Aqueous solutions of glucose and L-alanine were refluxed and, after specific periods of heating, the reaction was stopped by rapid cooling on ice, and the 2-osuloses and deoxyosones produced were then derivatized with 1,2-diaminobenzene at room temperature. Monitoring the effluent at 320 nm, the quinoxaline derivatives formed were separated by HPLC on RP-18 material as outlined in Fig. 3; they were then quantified by using the synthesized reference compounds as external standards. Thermal treatment of the glucose/L-alanine solution led to a drastic increase in the concentration of both the deoxyhexosones as well as the 2-hexosulose (Fig. 4). Already after 20 min of heating, the 2-hexosulose had reached a maximum concentration of about 50 μg/mmol glucose, whereas for 3-deoxy-2-hexosulose and 1-deoxy-2,3-hexodiulose, maximum concentrations of 1000 and 290 μg/mmol glucose, respectively, were determined after 40 min. Independently of the reaction time, the concentrations of 2-hexosulose and 1-deoxy-2,3-hexodiulose, respectively, were 20 times and 3 times lower than that of the 3-deoxy-2-hexosulose.

To study the influence of the carbohydrate moiety on the degradation of the sugars, the hexose was then substituted with the pentose, xylose. Heating an aqueous solution of xylose in the presence of L-alanine resulted in an increase in the concentrations of both the deoxypentosones and 2-pentosulose (Fig. 5). After 10 min of thermal treatment, the deoxyosones had already reached maximum concentrations of 1260 and 610 μg/mmol xylose corresponding to a yield of about 1.0% and 0.5% for the 3-deoxy-2-pentosulose and the 1-deoxy-2,3-pentodiulose, respectively. The maximum concentration of the 2-pentosulose amounted about

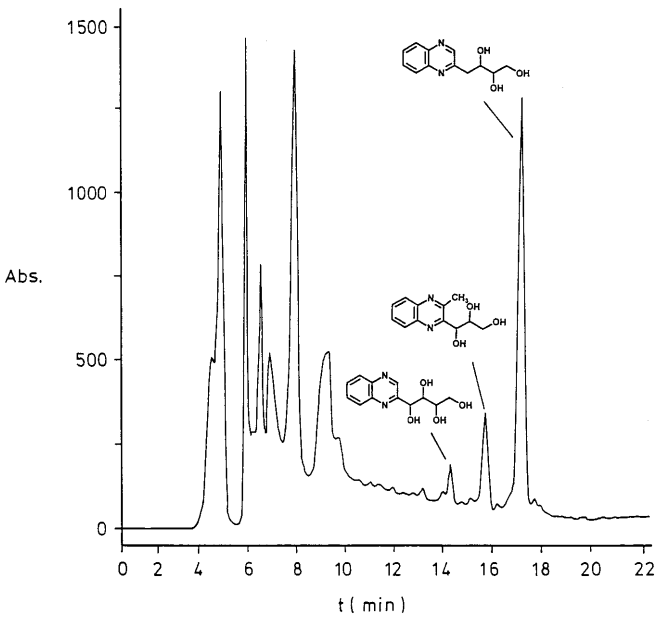

Fig. 3 RP-HPLC chromatogram of the quinoxalines of 2-hexosulose, 3-deoxy-2-hexosulose and 1-deoxy-2,3-hexodiulose formed in a heated aqueous solution of glucose and L-alanine. *Abs* Absorbance

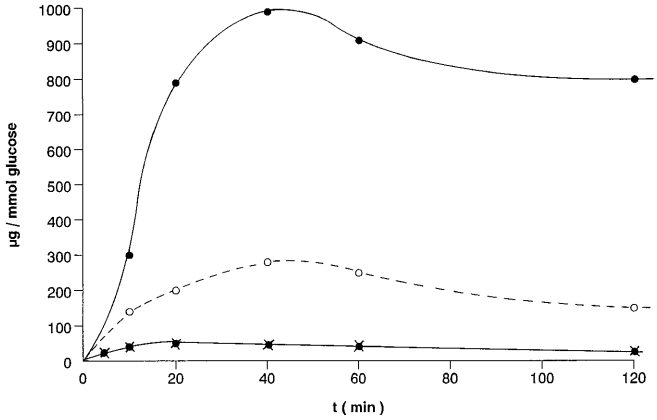

Fig. 4 Time course of the formation of 3-deoxy-2-hexosulose (—●—), 1-deoxy-2,3-hexodiulose (···○···) and 2-hexosulose (—◆—) in a heated aqueous solution of glucose and L-alanine

200 µg/mmol xylose, which was determined after 10 min of heating. Prolonging the reaction times led to a significant decrease in the concentrations of the deoxyosones and the 2-pentosulose.

It is well known from the literature [5] that deoxyosones and 2-osuloses are not stable and, due to dicarbonyl reactivity, can either be degraded to cyclic compounds with the carbon chain of the sugar intact, or be fragmented into short-chain carbonyl compounds upon C-C cleavage. We, therefore, focused the following quantitative studies on the formation of furans as well as C_2, C_3 and C_4 compounds in the carbohydrate/L-alanine mixtures. The formation of furan-2-carboxaldehyde, 5-(hydroxymethyl)furan-2-carboxaldehyde, the α-dicarbonyls glyoxal, 2-oxopropanal, butane-2,3-dione and the hydroxycarbonyls glycolaldehyde, hydroxy-2-propanone and 2-hydroxy-3-butanone was, therefore, quantitatively followed during the heating of aqueous solutions of xylose or glucose, in the presence of L-alanine. Because the carbohydrate fragmentation products are very reactive and hydrophilic, the dicarbonyls were derivatized with 1,2-diaminobenzene as stable quinoxalines, and the hydroxycarbonyls were converted with ethoxamine into their stable syn/anti-*O*-ethyloximes (Fig. 6). In order to guarantee exact quantitative data of these reactive carbohydrate degradation products, stable isotope dilution analyses were developed using [$^{13}C_4$]-butane-2,3-dione and [$^{13}C_4$]-3-hydroxy-2-butanone, respectively, as the internal standards for the α-dicarbonyl- and the hydroxycarbonyl compounds. Quantification was then performed by MS by scanning the pseudomolecular ions $[M+1]^+$ of the quinoxaline derivatives (Fig. 7) and the syn/anti-*O*-ethyloximes (Fig. 8) of the analytes and the isotope-labelled internal standards.

Heating of the glucose/L-alanine solution led to a drastic increase in the amount of glyoxal (Fig. 9). After only 10 min of heating a maximum concentration of 98 µg/mmol glucose was reached, corresponding to a yield of about 0.2%. In parallel with the decrease in glyoxal, the concentrations of the other carbohydrate degradation products increased, amongst which the C_3 compounds, hydroxy-2-propanone and 2-oxopropanal as well as 5-(hydroxymethyl)-furan-2-carboxaldehyde were the predominant compounds formed, e.g after 180 min, 10 times more hydroxy-2-propanone than glycolaldehyde had been formed, and twice as much 2-oxopropanal.

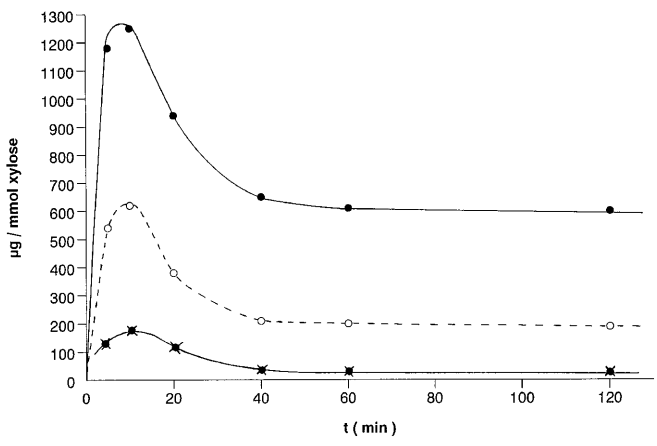

Fig. 5 Time course of the formation of 3-deoxy-2-pentosulose (—●—), 1-deoxy-2,3-pentodiulose (···○···) and 2-pentosulose (—◆—) in a heated aqueous solution of xylose and L-alanine

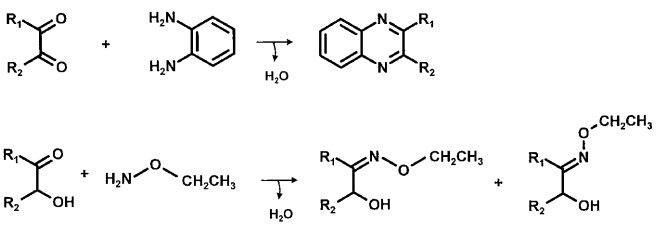

Fig. 6 Derivatisation of dicarbonyl and hydroxycarbonyl carbohydrate fragmentation products with 1,2-diaminobenzene and *O*-ethoxamine, respectively

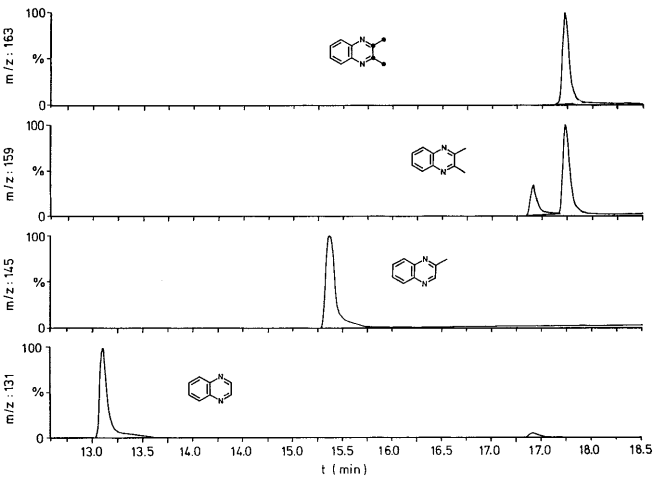

Fig. 7 Mass spectometry of quinoxaline derivatives of glyoxal, 2-oxopropanal, butane-2,3-dione and [$^{13}C_4$]-butane-2,3-dione

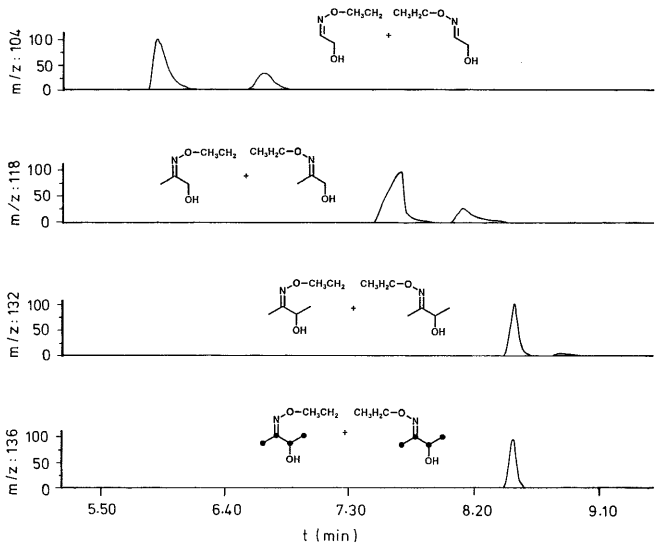

Fig. 8 Mass spectometry of syn/anti-*O*-ethyloximefig glycolaldehyde, hydroxy-2-propanone, 3-hydroxy-2-butanone and [$^{13}C_4$]-3-hydroxy-2-butanone

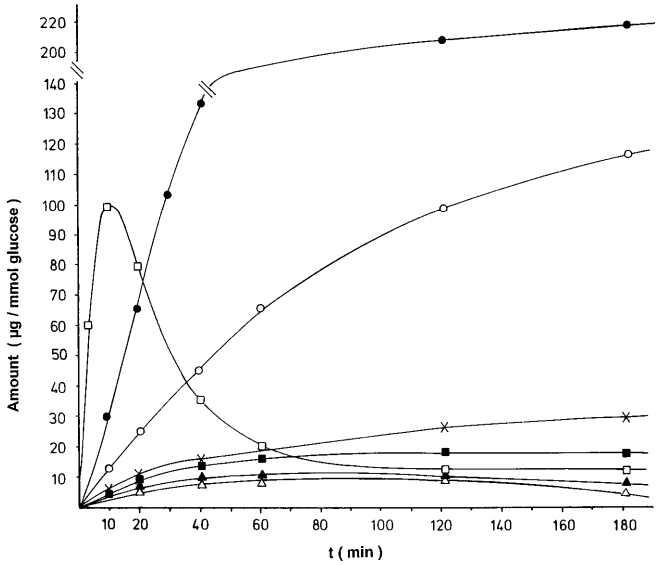

Fig. 9 Time course of the formation of carbohydrate degradation products in a thermally treated aqueous solution of glucose and L-alanine: glyoxal (□), 2-oxopropanal (○), butane-2,3-dione (△), glycolaldehyde (■), hydroxy-2-propanone (●), 2-hydroxy-3-butanone (▲), 5-(hydroxymethyl)furan-2-carboxaldehyde (×)

Also, heating an aqueous solution of xylose in the presence of L-alanine led to the preferential formation of glyoxal at the very beginning of the thermal treatment; after only 5 min, the glyoxal concentration reached a maximum of 59 µg/mmol xylose, corresponding to a yield of about 0.1% (Fig. 10). Prolonging the reaction time led to a drastic decrease in the amount of glyoxal; after 20 min its concentration was 15 times lower than at 5 min. In parallel with the decrease in glyoxal, the concentrations of the other dicarbonyl compounds, as well as of furan-2-carboxaldehyde, increased rapidly; the concentrations of 2-oxopropanal increased dramatically, reaching a yield of about 0.2% (120 µg/mmol xylose) after a reaction time of 180 min. Significant changes were also observed in the concentrations of the hydroxycarbonyl compounds. Whilst hydroxy-2-propanone increased rapidly with increasing heating time, glycolaldehyde had already reached its maximum concentration after 10 min. Over the complete heating period of 180 min, the concentration of 3-hydroxy-2-butanone was significantly lower than the concentrations of the other carbohydrate degradation products.

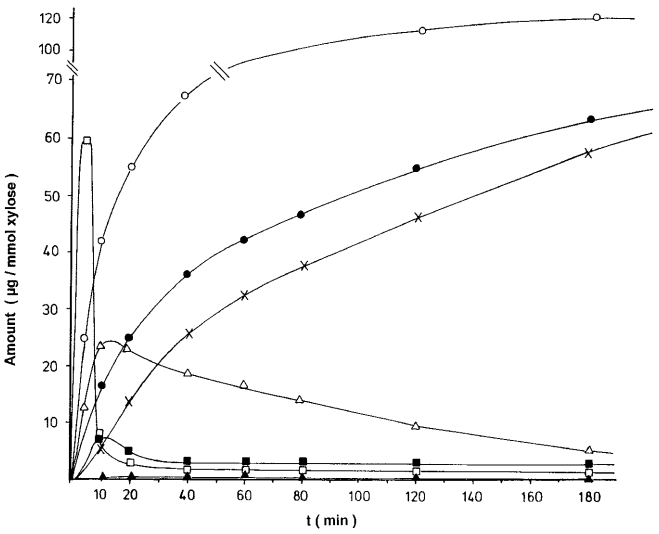

Fig. 10 Time course of the formation of carbohydrate degradation product in a thermally treated aqueous of xylose and L-alanine: glyoxal (□), 2-oxopropanal (○), butane-2,3-dione (△), glycolaldehyde (■), hydroxy-2-propanone (●), 2-hydroxy-3-butanone (▲), furan-2-carboxaldehyde (×)

These quantitative data give insights into the formation of reactive carbohydrate degradation products, and offer the possibility of evaluating the role of these reaction intermediates as browning precursors in the Maillard reaction.

Determination of the browning precursor activities of carbohydrate degradation products

In order to gain insights into their browning precursor activities (BPAs), the actual amount of each reaction intermediate in the heated carbohydrate/L-alanine solutions was related to its browning ability by combining chemical/instrumental techniques and visual/sensory analysis. We defined the BPA of a carbohydrate degradation product as the multiplication product of the concentration and the browning ability, as shown in Eq. 1:

$$\text{BPA} = \text{concentration (µmol/mmol)} \times \text{browning ability (CD factor)} \quad (1)$$

To determine browning abilities, binary mixtures of selected carbohydrate degradation products and L-alanine were recently heated in aqueous solution and the intensity of the browning developed was measured on the basis of CD factors [4]. The reaction mixtures were diluted step by step until a colour difference between the sample and two blanks could just be visually detected using a triangle test. Using this procedure, the carbohydrate degradation products were successfully ranked according to their browning abilities [4]. In the study presented here we also carried out comparative experiments with 2-xylosulose and 2-glucosulose. As shown in Table 1, glycolaldehyde and furan-2-carboxaldehyde were by far the most effective colour precursors in the presence of L-alanine, followed by 2-oxopropanal, 3-deoxy-2-pentosulose, 3-deoxy-2-hexosulose, glyoxal, 2-xylosulose and 2-glucosulose, for which somewhat lower CD factors were determined. In the presence of 5-(hydroxymethyl)furan-2-carboxaldehyde, hydroxy-2-propanone and 2-hydroxy-3-butanone, the heated aqueous L-alanine solution was nearly colourless.

Following Eq. 1, the browning precursor activities of the hexose degradation products were then calculated for different stages of the heating process. As shown in Table 2, the C_2 fragment, glyoxal, was found to have the highest browning activity in the glucose/L-alanine solution which was heated for only 5 min, followed by 3-deoxy-2-hexosulose and glycolaldehyde with 3- and 4.7-fold lower precursor activities. Increasing the reaction time led, however, to significant changes in the contribution of the hexose degradation products to the solution's colour. The participation of these reaction intermediates in the browning, especially that of 3-deoxy-2-hexosulose, increased during the first 10 min. A further prolongation of the reaction time resulted in a drastic decrease in the browning activity of glyoxal, whereas the contribution of 3-deoxy-2-hexosulose, glycolaldehyde and, especially, 2-oxopropanal, increased significantly. After 120 min of thermal treatment, 3-deoxy-2-hexosulose and 2-oxopropanal were found to be the most effective colour precursors.

Calculation of the BPAs of the pentose degradation products revealed that 3-deoxy-2-pentosulose was the most important browning precursor in the xylose/L-alanine solution which was reacted for 5 min (Table 3). Also, glyoxal and 2-xylosulose were found to be effective colour precursors in the presence of L-alanine, whereas the other pentose degradation products contributed only to a minor extent to the formation of browning substances. Increasing the reaction time led, however, to significant changes in the contribution of certain carbohydrate degradation products to the solution's colour. After 10 min of thermal treatment the precursor activity of glyoxal was already drastically

Table 1 Browning ability of carbohydrate degradation products in the presence of L-alanine (partly according to [4])[a]. *CD* Colour dilution

Carbohydrate degradation product	CD factor[b]
Glycolaldehyde	1024
Furan-2-carboxaldehyde	1024
2-Oxopropanal	256
3-Deoxy-2-pentosulose	256
3-Deoxy-2-hexosulose	128
Glyoxal	128
2-Xylosulose	128
2-Glucosulose	128
Butane-2,3-dione	64
2-Hydroxy-3-butanone	8
5-(Hydroxymethyl)furan-2-carboxaldehyde	2
Hydroxy-2-propanone	1

[a] Mixtures of carbohydrate degradation products (1 mmol) and L-alanine (1 mmol), dissolved in 5 ml phosphate buffer (0.5 mol/l, pH 7.0), were refluxed for 15 min

[b] To determine the CD factor the reaction mixture was diluted step by step (1 + 1, by vol.) until a colour difference between the sample (5 ml) and two blanks (tap water, 5 ml) in a glass vial (1 cm i.d.) could just be visually detected using a triangle test [17]

Table 2 Influence of the heating time on the relative browning activity of hexose degradation products in an aqueous solution of glucose and L-alanine

Hexose degradation product	Relative browning activity[a]			
	5 min	10 min	60 min	120 min
Glyoxal	161	219	44	26
3-Deoxy-2-hexosulose	55	237	727	635
Glycolaldehyde	34	68	273	288
2-Oxopropanal	25	45	231	352
2-Hexosulose	22	30	26	14
Butane-2,3-dione	1	3	13	12
Hydroxy-2-propanone	<1	<1	2	3
2-Hydroxy-3-butanone	<1	<1	1	1
5-(Hydroxymethyl)furan-2-carboxaldehyde	<1	<1	<1	<1

[a] The relative browning activity was calculated by multiplicating the concentration with the browning effectivity (CD factor) for each compound

Table 3 Influence of the heating time on the relative browning activity of pentose degradation products in an aqueous solution of xylose and L-alanine

Hexose degradation product	Relative browning activity[a]			
	5 min	10 min	60 min	120 min
3-Deoxy-2-pentosulose	2304	2438	1192	1182
Glyoxal	133	18	5	5
2-Oxopropanal	89	148	276	386
Glycolaldehyde	68	137	60	58
2-Xylosulose	116	158	20	16
Butane-2,3-dione	35	34	25	16
Furan-2-carboxaldehyde	20	53	351	485
Hydroxy-2-propanone	<1	<1	<1	<1
2-Hydroxy-3-butanone	<1	<1	<1	<1

[a] The relative browning activity was calculated by multiplicating the concentration with the browning effectivity (CD factor) for each compound

lower, whereas the browning activity of 3-deoxy-2-pentosulose, 2-xylosulose, 2-oxopropanal and glycolaldehyde had increased significantly. Prolonging the reaction time to 60 min led again to a decrease in the contribution of 3-deoxy-2-pentosulose, glycolaldehyde and, especially, of 2-xylosulose, to the colour of the solution whereas the participation of furan-2-carboxaldehyde and 2-oxopropanal in the browning reactions was strongly increased. This effect was even more drastic after 120 min. Although hydroxy-2-propanone was formed in high concentrations (Fig. 10), this C_3 compound was not significantly involved in the browning reaction with L-alanine.

Discussion

The data presented indicated that the application of appropriate quantification techniques offers the possibility of following the time course of the formation of reaction intermediates formed from carbohydrates and amino acids. Independently of the carbohydrate moiety, 2-osuloses and glyoxal were formed from glucose as well as xylose, predominantly at the very beginning of the thermal treatment. This is in good agreement with the favoured formation of 2-osuloses from carbohydrates and amino acids prior to the Amadori rearrangement and its proposed fragmentation upon the liberation of glyoxal [2–4]. With increasing reaction times, the formation of 1- and 3-deoxyosones, 2-oxopropanal and hydroxy-2-propanone from xylose and glucose was also favoured. In good agreement with data reported recently by Weenen and Apeldoorn [11], heating of the pentose liberated the 2-oxopropanal more effectively than the hexose. In addition, high yields of furan-2-carboxaldehyde and 5-(hydroxymethyl)furan-2-carboxaldehyde were produced from the pentose and the hexose, respectively. Except at the beginning of the Maillard reaction, the fragmentation of the pentose as well as the hexose carbon chain in C_2 and C_4 compounds was demonstrated to be of only minor importance in carbohydrate degradation.

The formation of deoxyosones and short-chain dicarbonyls via carbohydrate degradation has been investigated previously, however, in most studies the Maillard mixtures were reacted in the presence of surplus amounts of trapping agents, such as 1,2-diaminobenzene [7–10, 13] or aminoguanidine [18, 19], in order to transform the reactive dicarbonyl intermediates in situ into stable quinoxaline or triazine derivatives. But, in using these in situ derivatization techniques, dicarbonyls are removed from reaction equilibria and, therefore, certain Maillard reaction pathways are quantitatively influenced. Also, competitive reactions between the amino-group-containing trapping agents and the amino acid with the carbohydrate might significantly influence the yields of carbohydrate degradation products. This in situ derivatization do not, therefore, lead to accurate quantitative data. Because the stable derivatives formed are not degraded, this technique leads to an accumulation of the derivatives and, therefore, does not offer insights into the changes in the actual concentrations of these compounds. The formation of the reactive 2-oxopropanal upon heating of glucose was, therefore, recently investigated by derivatizing the dicarbonyl formed after certain reaction times [11, 12]. To achieve more reliable results in the present investigation stable isotope dilution analysis was applied in order to quantify the reactive dicarbonyls formed.

Similarly for the dicarbonyls, data on the actual amounts of hydroxycarbonyl compounds were not available in the literature. In the present investigation, a method employing stable isotope dilution analysis was, therefore, developed to enable a study of the time course of the production of hydroxycarbonyl intermediates.

To elucidate the role of these carbohydrate degradation products in non-enzymatic browning of carbohydrate/L-alanine solutions, the BPA was calculated for each compound by multiplying its concentration by its browning ability (CD factor). At the very beginning of the thermal treatment, 3-deoxyosone, glyoxal and glycolaldehyde were shown to have the highest BPAs in a carbohydrate/L-alanine solution. In contrast to glucose, 2-oxopropanal was found to be an effective colour precursor even at the beginning of heating xylose in the presence of L-alanine. A prolongation of the reaction time led, independently of the carbohydrate moiety, to a drastic decrease in the precursor activity of glyoxal and, in parallel, to an increase in the participation of 2-oxopropanal and also furan-2-carboxaldehyde in the browning reactions. Hayashi and Namiki [6] also observed a rapid browning of an aqueous solution containing glycolaldehyde as well as 2-oxopropanal when heated in the presence of β-alanine. Because the authors did not, however, correlate the measured browning intensities with the actual concentrations of the reaction intermediates present in the carbohydrate/amino acid mixtures, they were not able to evaluate the role of these carbohydrate fragments in non-enzymatic browning or gain insights into the changes in browning precursors during the heating process. In the present paper, certain carbohydrate degradation products could be evaluated as browning precursors, and it could be successfully demonstrated that the activity of these reaction intermediates in producing browning substances changes during thermal treatment.

Conclusions

Correlating chemical/analytical with sensory data gives insights into the role of carbohydrate degradation products in non-enzymatic browning and offers the possibility of identifying the key browning precursors leading to the development of colour in mixtures of carbohydrates and amino acids. Information on these browning precursors might be very helpful with respect to con-

trolling and optimizing the non-enzymatic browning occurring during food processing in order to improve the quality of foods more efficiently.

Acknowledgements This work was funded by grants from the Deutsche Forschungsgemeinschaft. I am grateful to Mr. J. Stein for his excellent technical assistance.

References

1. Kuhn R, Weygand F (1937) Ber Dtsch Chem Ges B70:769–772
2. Kawakishi S, Uchida K (1990) Autoxidation of Amadori compounds in the presence of copper ion and its effects on the oxidative damage of protein. In: Finot PA, Aeschbacher HU, Hurrell RF, Liardon R (eds) Maillard reaction in food processing, human nutritition and physiology. Birkäuser, Basel, pp 475–480
3. Kawakishi S, Tsunehiro J, Uchida K (1991) Carbohydr Res 211:167–171
4. Hofmann T, Bors W, Stettmeier KJ (1999) J Agric Food Chem 47:379–390
5. Ledl F, Schleicher E (1992) Angew Chem 102:597–626
6. Hayashi T, Namiki M (1986) Agric Biol Chem 50:1965–1970
7. Morita N, Inoue K, Takagi M (1981) Agric Biol Chem 45:2665–2668
8. Morita N, Inoue K, Takagi M (1985) Agric Biol Chem 49:3279–3289
9. Beck J, Ledl F, Severin T (1988) Carbohydr Res 177:240–243
10. Nedvidek W, Ledl F, Fischer P (1992) Z Lebensm Unters Forsch 194:222–228
11. Weenen H, Apeldoorn W (1996) Carbohydrate cleavage in the Mailland reaction. In: Taylor AJ, Mottram DS (eds) Flavour science: recent developments. Royal Society of Chemistry, Cambridge, pp 211–216
12. Homoki-Farkas P, Örsi F, Kroh LW (1997) Food Chem 59:157–163
13. Hollnagel, Kroh (1998) Z Lebensm Unters Forsch 207:50–54
14. Hofmann T (1998) Carbohydr Res 313:215–224
15. Salomon L, Burns J, King C (1952) J Am Chem Soc 74:5161
16. Schieberle P, Hofmann T (1997) J Agric Food Chem 45:227–232
17. Hofmann T (1998) Carbohydr Res 313:203–213
18. Hirsch J, Petrakova E, Feather MS (1992) Carbohydr Res 232:125–130
19. Hirsch J, Mossine VV, Feather MS (1995) Carbohydr Res 273:171–177