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Characterization of Model Melanoidins by the Thermal Degradation Profile

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Different types of model melanoidins were thermally degraded, with subsequent identification of the volatiles produced, to obtain and compare the thermal degradation profile of various melanoidins. At first, the volatiles produced from heated glucose/glycine standard melanoidins were compared with glucose/glutamic acid and L-(+)-ascorbic acid/glycine standard melanoidins. In the headspace of heated glucose/glycine melanoidins, mainly furans, were detected, accompanied by carbonyl compounds, pyrroles, pyrazines, pyridines, and some oxazoles. Heating of L-(+)-ascorbic acid/glycine melanoidins resulted in relatively more *N*-heterocycles, while from glucose/glutamic acid melanoidins no *N*-heterocycles were formed. In a second part, a chemical treatment was applied to glucose/glycine melanoidins prior to the thermal degradation. Acid hydrolysis was performed to cleave glycosidically linked sugar moieties from the melanoidin skeleton. Nonsoluble glucose/glycine melanoidins were also subjected to an oxidation. The results indicate that the thermal degradation profile is a useful tool in the characterization of different types of melanoidins.

KEYWORDS: Maillard reaction; melanoidins; characterization; degradation; pyrolysis

INTRODUCTION

The Maillard reaction includes a complex series of reactions between reducing sugars and compounds possessing a free amino group. End products of the reaction sequence are flavor compounds on one hand, and brown colored nitrogenous polymers, called melanoidins, on the other hand. Until now, despite extensive studies, it has not been possible to isolate or completely characterize a single melanoidin, if possible at all, due to their extremely complex and heterogeneous nature (*1*). They are, however, important ingredients of the human diet, for example, in coffee, malt, or bakery products, since they are formed during the cooking, processing, and storage of food.

Because of the complexity of real food systems, most studies on melanoidins have focused on model browning reactions. The most widely studied model system is the glucose/glycine model system (*2*). To allow a comparison between results obtained from different laboratories, a standardized protocol has been developed as part of a European Research Program (COST Action 919), in which standard glucose/glycine melanoidins are prepared (*3*). The term COST refers to European Cooperation in the field of Scientific and Technical Research, with a geographical scope beyond the European Union.

Whenever melanoidins are heated, volatiles are produced, which contribute largely to the aroma of roasted or baked food products (*4*). In previous research by our research group, both nondialyzable and nonsoluble standard COST glucose/glycine melanoidins were heated at temperatures ranging from 100 to 300 °C (temperatures relevant for food preparation) and the volatiles produced were identified (*5*).

In a continuation of this research, the same thermal degradation procedure was now applied to different types of melanoidins. On one hand, melanoidins prepared with glucose and glutamic acid were thermally degraded, as well as melanoidins prepared with L-(+)-ascorbic acid and glycine, to compare the volatiles produced with the volatiles from standard COST glucose/glycine melanoidins. Thus, the influence of the carbonyl compound and the amino compound in the Maillard reaction, on the thermal degradation profile of the resulting melanoidins, was investigated.

On the other hand, a chemical treatment was applied to the melanoidins prior to the thermal degradation. A chemical hydrolysis was performed to release glycosidically linked sugar moieties, which were recently shown to be present, from the melanoidin skeleton (*6*). Apart from this, an oxidation of the melanoidins was performed. This oxidation process may result in more reactive subunits that have a pivotal role in flavor generation.

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MATERIALS AND METHODS

Materials. Glycine (>99%) and D-glucose (99%) were obtained from Sigma Chemical Co. (Bornem, Belgium). Dialysis tubing with a flat width of 33 mm was purchased from Sigma (Bornem, Belgium). This cellulose membrane retains >90% cytochrome *c* (MW 12 400) in solution over a 10 h period. The dialysis tubing was prepared according to the manufacturer's instructions.

Preparation of Melanoidins. In a 300-mL Christ filter bottle 0.05 mol of D-glucose (9.00 g) or L-(+)-ascorbic acid (8.80 g), and 0.05 mol of glycine (3.75 g) or glutamic acid (7.35 g) were dissolved in 20 mL of distilled water. The solution was frozen in a bath of liquid nitrogen. Subsequently, it was freeze-dried (Christ alpha 1−4) until all the water had been removed (i.e., constant weight). The carbonyl compound−amino acid mixture was placed in an oven (Memmert), which was equipped with a fan and had been preheated to and stabilized at 125 °C. The mixture was heated for exactly 2 h without covering. After heating, the filter bottle was allowed to cool to room temperature in a desiccator. The solid was transferred to a mortar and carefully ground to a fine powder. Five grams of the ground material was added to 200 mL of distilled water and the solution was stirred for 12 h to dissolve as much material as possible. This suspension was filtered through Whatman No. 4 filter paper, and the filtrate, which contained the water-soluble melanoidins, was collected. The residue on the filter paper was washed with 2 × 20 mL of distilled water. The combined filtrate and washings are made up to 250 mL with distilled water. This mixture is called solution A. The residue obtained, the so-called water-insoluble fraction of the melanoidins, was frozen, freeze-dried, and stored at −32 °C until further use.

Isolation of Nondialyzable Melanoidins by Dialysis. Fifty milliliters of the soluble melanoidin solution A was brought in 21 cm of dialysis tubing and was dialyzed against 1 L of distilled water for 24 h at 4 °C with four changes of the surrounding water. At the end of the dialysis, the contents of the dialysis tubing with the high molecular weight fraction (HMW) or so-called nondialyzable melanoidins, were transferred to a 500-mL round-bottom flask, frozen in a liquid nitrogen bath and freeze-dried until all the water had been removed. If the low molecular weight fraction (LMW) of the melanoidins was needed, the dialysate resulting from the first change of water was collected and freeze-dried.

Acid Hydrolysis. Acid hydrolysis was performed according to the optimal conditions found by Cämmerer et al. (*6*). Sufficient amounts of melanoidins were dissolved or suspended (in the case of nonsoluble melanoidins) in acid solution to a concentration of 20 mg/mL. The mixture was heated in an oil bath, and stirred for 1 h. After rapid cooling of the sample in an ice bath, 1 mL of distilled water was added for every 1 mL of solution. The mixture was neutralized using a 2 N NaOH solution and filtered. The water-insoluble residue on the filter was washed with distilled water and dried (called fraction "N"). The filtrate was subjected to dialysis, as described higher, leading to an HMW and an LMW fraction. For the acid hydrolysis, three combinations of concentration and temperature were used: (1) 1 N HCl, 1 h, room temperature (Hydr1); (2) 1 N HCl, 1 h, reflux (Hydr2); (3) 36 N H_2SO_4, 1 h, reflux conditions (Hydr3).

Oxidation. Oxidative degradation of melanoidins was done according to Kato and Tsuchida (*7*). Five grams of nonsoluble glucose/glycine melanoidins was suspended in 2 N K_2CO_3 (80 mL) and 3% $KMnO_4$ (800 mL) was gradually added, while stirring at 30 °C. The reaction mixture was heated at 60 °C until decolorization and filtered. For the oxidation with periodate, 5 g of nonsoluble glucose/glycine melanoidins was suspended in H_2O (80 mL), 0.5 M $NaIO_4$ (200 mL) was added, and the mixture was allowed to react for 4 h at room temperature.

SPME. Silanized 4-mL SPME-vials (Supelco Inc., Bellefonte, USA) filled with 50 mg of melanoidins were covered with PTFE-silicone septa and open top polypropylene (Supelco) closures and heated on a sand bath to maintain a constant temperature (± 10 °C) during 10 min. After cooling of the vials to room temperature, the SPME fiber (DVB/Carboxen/PDMS, Supelco Inc., Bellefonte, USA) was exposed to the headspace of the heated melanoidin during 5 min.

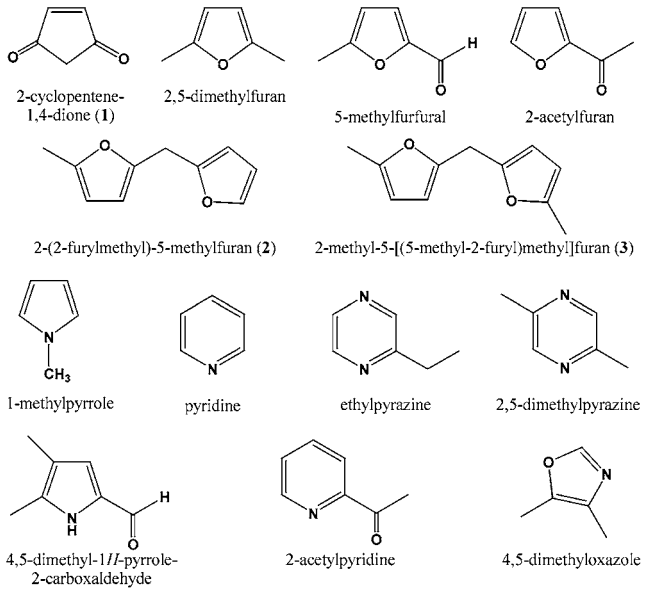

Figure 1. Structures of some representative volatiles identified in the headspace of heated melanoidins.

Mass Spectrometry. For the analysis of the SPME-extracts a Hewlett-Packard 6890 GC Plus coupled with a HP 5973 MSD (Mass Selective Detector-Quadrupole type), equipped with a CIS-4 PTV (Programmed Temperature Vaporization) Injector (Gerstel), and a HP5-MS capillary column (30 × 0.25 mm i.d.; coating thickness 0.25 μm) was used. Working conditions were injector 250 °C, transfer line to MSD: 250 °C, oven temperature: start 40 °C, hold 2 min; programmed from 40 to 120 °C at 4 °C min^{-1} and from 120 to 240 °C at 30 °C min^{-1}, hold 2 min; carrier gas (He) 1.2 mL min^{-1}; splitless; ionization EI 70 eV; acquisition parameters: scanned *m*/*z* 40−200 (0−10 min), 40−300 (10−20 min), 40−400 (> 20 min). Substances were identified by comparison of their mass spectra and retention times with those of reference substances and by comparison with the Wiley (6th) and the NIST Mass Spectral Library (Version 1.6d, 1998). When only MS data were available, identities were considered to be tentative.

RESULTS AND DISCUSSION

HMW water-soluble melanoidins were obtained from the model systems glucose/glycine, glucose/glutamic acid, and L-(+)-ascorbic acid/glycine in a molar ratio of 1:1 in anhydrous medium at 125 °C (Materials and Methods). Water-insoluble melanoidins were obtained only from glucose/glycine melanoidins and in very small amounts from L-(+)-ascorbic acid/glycine melanoidins. This indicates that for glucose/glycine melanoidins, the polymerization reaction occurs much faster than when glutamic acid is used, since only soluble melanoidins are formed in the reaction of glucose with glutamic acid.

For each melanoidin fraction, 50 mg was heated on a sand bath at a constant temperature of 200 or 250 °C. After cooling, the headspace was sampled by means of SPME and analyzed by GC-MS. The volatiles formed, were divided in six main groups based on the chemical structure: carbonyl compounds, furans, pyrroles, pyridines, pyrazines, and oxazoles, as has been discussed in the previous paper (*5*). In **Figure 1**, some representative structures of compounds identified in the headspace of heated melanoidins are depicted. Using this subdivision, a thermal degradation profile of each melanoidin fraction was composed. For this purpose, for each group of compounds, the share in terms of percentage of the total GC peak area is depicted in bar graphs (**Figures 2−5**). The total GC peak areas are shown in **Tables 1** and **2**. The error bars in the graphs represent twice

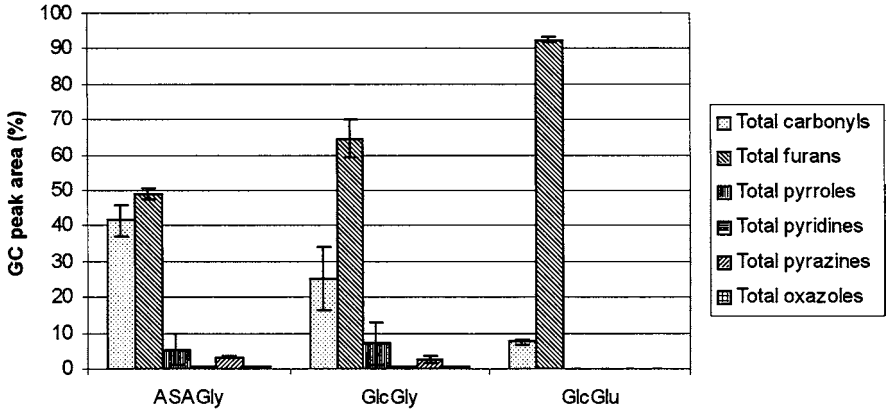

Figure 2. Thermal degradation profile of HMW water-soluble melanoidins prepared from D-glucose/glycine (GlcGly), L-(+)-ascorbic acid/glycine (ASAGly), and D-glucose/glutamic acid (GlcGlu) heated at 200 °C.

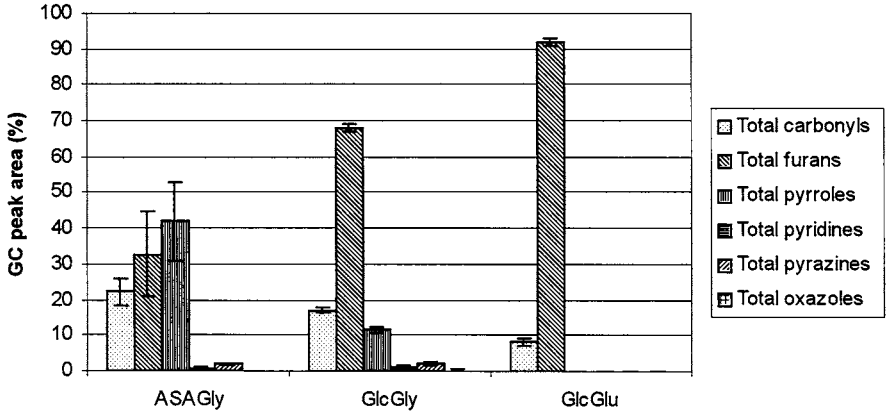

Figure 3. Thermal degradation profile of HMW water-soluble melanoidins prepared from D-glucose/glycine (GlcGly), L-(+)-ascorbic acid/glycine (ASAGly), and D-glucose/glutamic acid heated at 250 °C.

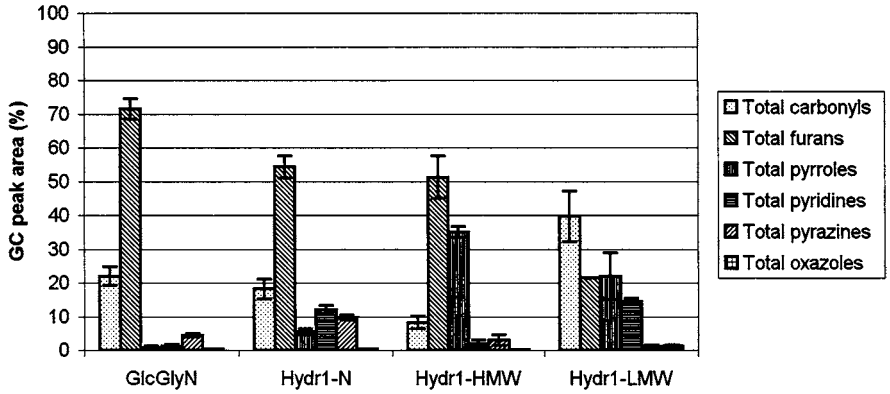

Figure 4. Thermal degradation profile (heating at 250 °C) of different melanoidin fractions (N: nonsoluble, HMW: high molecular weight, LMW: low molecular weight), obtained from nonsoluble glucose/glycine melanoidins, before (GlcGlyN) and after acid hydrolysis, type 1 (1 N HCl, 1 h, rt) (Hydr1).

the standard deviation of three replications. Though the relative standard deviation (RSD) on absolute values of peak areas is sometimes high (**Tables 1** and **2**), for the amounts relative to the total peak area, the average RSD lies at 32 ± 7%. Considering the nature of the experiments, this is acceptable, and will be taken into account for the conclusions of the experiments.

First, the thermal degradation profiles of HMW water-soluble melanoidins obtained from the different model systems D-glucose/glycine (GlcGly), L-(+)-ascorbic acid/glycine (ASAGly), and D-glucose/glutamic acid (GlcGlu) were compared. Each melanoidin fraction was heated and analyzed in triplicate (**Figure 2**, heating at 200 °C, and **Figure 3**, heating at 250 °C). The total GC peak areas obtained after GC-MS analysis of the headspace extracts are shown in **Table 1**. Clear differences can be observed in the composition of the headspace extract of the different melanoidins; therefore, these thermal degradation profiles can be used to characterize different kinds of melanoidins.

The carbonyl compounds include acyclic C-4 and C-5 mono- and dicarbonyls, 2-methyl-2-cyclopenten-1-one and 2-cyclopentene-1,4-dione. The amounts of carbonyl compounds detected are quite constant for the different melanoidins, and have little specificity. Furans are important in the composition of the volatiles for all types of melanoidins, but especially the azaheterocyclic compounds define the difference. For these nitrogen-containing compounds, production is substantially higher at 250 °C than at 200 °C. Therefore, the thermal

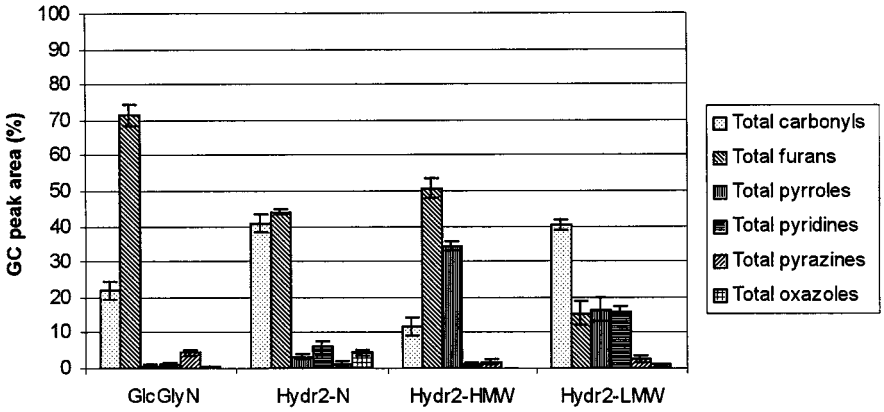

Figure 5. Thermal degradation profile (heating at 250 °C) of different melanoidin fractions (N: nonsoluble, HMW: high molecular weight, LMW: low molecular weight), obtained from nonsoluble glucose/glycine melanoidins, before (GlcGlyN) and after acid hydrolysis, type 2 (1 N HCl, 1 h, reflux) (Hydr2).

Table 1. Average and Relative Standard Deviation (%) of the Total GC Peak Area ($\times 10^8$) Obtained after Thermal Degradation at Different Temperatures of Glucose/Glycine (GlcGly), Glucose/Glutamic Acid (GlcGlu) and L-(+)-Ascorbic Acid/Glycine (ASAGly) HMW Melanoidins

	200 °C		250 °C	
HMW melanoidins	average	RSD (%)	average	RSD (%)
GlcGly	6.8	49.4	5.2	62.0
GlcGlu	7.2	19.0	3.6	26.6
ASAGly	4.0	70.0	2.0	49.3

Table 2. Average and Relative Standard Deviation (%) of the Total GC Peak Area ($\times 10^8$) Obtained after Thermal Degradation at 250 °C of Different Melanoidin Fractions before and after Acid Hydrolysis of Nonsoluble Glc/Gly Melanoidins

type of hydrolysis	melanoidin fraction	average	RSD (%)
before hydrolysis	nonsol	1.26	21.5
1 N HCl, 1 h, rt	nonsol	0.89	28.9
	HMW	4.28	22.2
	LMW[a]	2.76	33.7
1 N HCl, 1 h, reflux	nonsol	0.18	57.2
	HMW	1.58	56.3
	LMW[a]	2.90	16.1

[a] 150 mg were heated, the exact amount of melanoidins is not known due to the presence of salt (NaCl from neutralization).

degradation profiles at 250 °C are most useful to differentiate between the different melanoidins.

The thermal degradation profile of standard HMW glucose/glycine melanoidins showed mainly furans (65 ± 5% at 200 °C, 55 ± 3% at 250 °C). Pyrroles counted for 7 ± 6% of the headspace profile at 200 °C, but amounted to 24 ± 7% when the melanoidins were heated at 250 °C. Pyrazines, pyridines, and oxazoles were found, but were quantitatively of minor importance in the headspace profile.

In the headspace of heated L-(+)-ascorbic acid/glycine melanoidins, besides furans, especially pyrroles were important. The amount of pyrroles released after heating the melanoidins increased strongly with heating temperature. An average of 42 ± 11% of the volatiles detected after heating of L-(+)-ascorbic acid/glycine melanoidins at 250 °C were pyrroles. Thermal degradation of L-(+)-ascorbic acid as such has been shown to yield mostly furan derivatives and α,β-unsaturated ketones with a five-membered ring (*8*). These are found among the furans and carbonyl compounds detected.

On the other hand, in the headspace of heated glucose/glutamic acid melanoidins, only furans and carbonyl compounds were found, but in high amounts. 2-Cyclopentene-1,4-dione (**1**) was quantitatively the most important compound among the carbonyl compounds detected. About 80% (79 ± 2%) of the headspace profile was composed of four major furan compounds: 2-methylfuran, 2,5-dimethylfuran, furfural, and 5-methylfurfural. No nitrogen-containing compounds could be identified after heating of glucose/glutamic acid melanoidins.

It is known that glutamic acid has a low reactivity in the formation of volatile azaheterocyclic compounds. In a comparison of the reactivity of different amino acids in the formation of azaheterocyclic compounds upon heating, glutamic acid was shown to be the lowest contributor to flavor formation among the tested amino acids in the presence of labeled glycine (*9*). In an investigation for the thermal degradation of amino acids, very little ammonia was released when glutamic acid was heated, in contrast to the easy deamination of most amino acids, among which the very similar aspartic acid (*10*). Glycine, on the contrary, having no side chain, is more flexible than other amino acids, and therefore highly reactive. This explains why from glucose/glycine melanoidins and from L-(+)-ascorbic acid/glycine melanoidins, many nitrogen-containing heterocycles were produced, and none from glucose/glutamic acid melanoidins under the same circumstances.

In the headspace of heated glucose/glutamic acid melanoidins relatively large amounts of bridged furan derivatives with a methylene unit as spacer were detected, indicating the presence of nonglycosidically linked sugar derivatives in the melanoidin skeleton. These bifurylmethane derivatives, such as 2-(2-furylmethyl)-5-methylfuran (**2**) and 2-methyl-5-[(5-methyl-2-furyl)methyl]furan (**3**) (depicted in **Figure 1**), were also found, though in lower amounts, in the headspace of heated glucose/glycine melanoidins, but not at all when melanoidins prepared with L-(+)-ascorbic acid were heated. Their presence also indicates that glucose residues are linked during the polymerization reaction, without incorporation of the amino acid or its degradation products in the cross-linking. Furaldehydes, formed after thermal treatment, probably result partly from heating of this type of structures.

In a second approach, glucose/glycine melanoidins were subjected to a chemical treatment, prior to the thermal degradation. The reflection of the chemical changes induced in the melanoidin structure, in the thermal degradation profile was investigated.

At first, an acid hydrolysis was carried out, to remove glycosidically linked sugar moieties, which form side chains

on the melanoidin skeleton (*6*). Acid-catalyzed hydrolysis of melanoidins was carried out using the optimal circumstances found by Cämmerer et al. (*6*), namely, 1 h reflux in 1 N HCl solution. In addition, a "soft" hydrolysis was performed, with 1 h reaction at room temperature in a 1 N HCl solution, and a very strong hydrolysis procedure, consisting of 1 h heating in concentrated H_2SO_4. It is expected that these hydrolytic conditions will result in other degradative actions, besides the cleavage of glycosidically linked sugars.

These three hydrolysis procedures were first applied to the nonsoluble fraction of standard glucose/glycine melanoidins. The thermal degradation profile of untreated nonsoluble glucose/glycine melanoidins is shown as the left bar graphs ('GlcGlyN') in **Figures 4** and **5** (heating at 250 °C, heating at 200 °C not shown). The majority (64 ± 4% at 200 °C, 72 ± 3% at 250 °C) of the volatiles produced from heating nonsoluble glucose/glycine melanoidins were furans. Little pyrroles are formed upon heating: pyrazines are the most important of the azaheterocyclic compounds (3.7 ± 0.8% at 200 °C, 4.4 ± 0.6% at 250 °C).

After hydrolysis and subsequent neutralization, the residue was separated in a nonsoluble and a soluble fraction by filtration, and the filtrate was again separated by dialysis in an HMW and an LMW fraction. The weight decrease after hydrolysis was noted as a measure for the breakdown of the structure. Hydrolysis with 1 N HCl, at room temperature and under reflux conditions, degraded 11 and 58% of the nonsoluble melanoidins into soluble compounds, respectively, while after treatment with 36 N H_2SO_4, no nonsoluble fraction was left. This shows an important weight decrease of nonsoluble melanoidins upon acid hydrolysis, even though these polymers usually show a very high resistance to many treatments (chemical and other) and are insoluble in common organic solvents.

Each fraction resulting from the hydrolysis procedures was subjected to thermal degradation at 200 °C once and at 250 °C in triplicate. The results for heating at 200 °C are not shown, the results for heating at 250 °C after acid hydrolysis, type 1 (1 N HCl, room temperature) and type 2 (1 N HCl, reflux conditions) are shown in **Figures 4** and **5**, respectively. **Table 2** shows the total GC peak areas obtained after heating of the different fractions.

The thermal degradation profile of the nonsoluble fraction after hydrolysis showed a decreasing importance of the furans, and an increasing importance of azaheterocyclic compounds. Quantitatively little volatiles were released upon heating, indicating a rigid structure left after hydrolysis (**Table 2**). In the HMW-melanoidins obtained by chemical hydrolysis of nonsoluble melanoidins, especially furans and pyrroles, were formed upon heating. In the LMW-fraction, thermal degradation gave rise to the formation of comparable amounts of furans, pyrroles, and pyridines. The results of both hydrolysis procedures are very comparable.

Hydrolysis type 3, with H_2SO_4, gave a difficult workup, and no dialysis was performed. In the thermal degradation profile of the soluble fraction (data not shown), furans only counted for 14 and 10%, when heated at 200 and 250 °C, respectively. Pyrroles (55 and 46%) were the most important group of compounds, followed by the pyridines (32 and 38%).

One would expect that the presence of cleaved sugar residues would lead to furan derivatives in the headspace of the heated LMW-fraction. However, mostly pyrroles were formed. A 2-fold explanation for this can be given. On one hand, it is known that the amount of cleaved sugars is rather limited. For HMW glucose/glycine melanoidins, Cämmerer et al. (*6*) found that 2.9 mg of glucose was cleaved for every 100 mg of melanoidins. This is a relatively low amount, which indicates that most of the sugar has been incorporated in the melanoidin skeleton after reaction, and is, as a consequence, visible in terms of furans upon heating. Since in our experiments, a weight decrease was found of 45 mg/100 mg of HMW melanoidins under the same conditions, this also implies that a significant amount of other residues is cleaved off from the melanoidin skeleton. On the other hand, it needs to be considered that the reaction conditions applied (acid solution, 1 h) are quite drastic, and that the cleaved sugar residues can react with other fragments in solution. When the melanoidins are prepared, glucose and glycine react under water-free conditions. Although a close contact between the reactants is pursued by a dissolution followed by a lyophilization, the question remains whether the contact between the reactants is sufficient. In any case, a reaction between sugar derivatives and nitrogen-containing melanoidin fragments in the acid solution during the hydrolysis procedure is probable. Reaction in solution of the melanoidin degradation products with nitrogen-containing fragments may be responsible for the formation of relatively more azaheterocyclic compounds upon heating, after hydrolytic degradation.

To investigate which volatiles present in the thermal degradation profile of acid-treated melanoidins, could result from glucose residues, an acid hydrolysis (1 N HCl, 1 h, rt) with subsequent thermal degradation of glucose was performed. Alcohols, ketones, and alkenes could be detected after heating, especially butanol, cyclopentanol, and cyclopentene derivatives. Therefore, only a neglectable amount of the volatiles detected upon heating of the melanoidin fractions after hydrolysis, results directly from glucose, and as a consequence the amount of free glucose in the mixture, obtained after acid hydrolysis of glucose melanoidins, is very low.

Hydrolysis with 1 N HCl (1 h, reflux conditions) was also applied to the HMW-fraction of standard glucose/glycine melanoidins, as well as to glucose/glutamic acid and ascorbic acid/glycine melanoidins (data not shown). For HMW glucose/glycine melanoidins, a weight decrease of 45% was found. The weight decrease was larger, 80 and 90%, respectively, when ascorbic acid/glycine and glucose/glutamic acid melanoidins were hydrolyzed. As discussed earlier, a fast polymerization takes place in the reaction of glucose with glycine, leading to a strong melanoidin network, difficult to degrade. This is less the case in the reactions of model component mixtures of glucose/glutamic acid and ascorbic acid/glycine. In these cases, no low molecular weight fractions were analyzed.

Hydrolysis of HMW glucose/glycine melanoidins gave results similar to those obtained from nonsoluble glucose/glycine melanoidins: thermal degradation of the HMW fraction left after hydrolysis, yielded more azaheterocyclic compounds, especially pyridines, but less furans. The HMW residue after acid hydrolysis of glucose/glutamic acid melanoidins yielded only carbonyls and furans upon heating, as expected, but also for ascorbic acid/glycine melanoidins, furans accounted for 88% of the headspace profile after hydrolysis, indicating that most nitrogen-containing compounds are cleaved of after hydrolysis (data not shown).

The LMW-fraction obtained after dialysis in the standard procedure for preparation of glucose/glycine melanoidins, contains unreacted starting material. Heating at 250 °C of the dried LMW-fraction results in further Maillard reaction with formation of a very dark porous structure. Analysis of the resulting volatiles showed mainly pyrroles (54 ± 3%), furans (23 ± 3%), and pyrazines (11 ± 4%). This reaction was not noticed when LMW-fractions resulting from hydrolysis of the

nonsoluble fractions were heated. Thus, the remaining amounts of cleaved sugar and amino acid in these fractions are not in those amounts that reaction can be visually observed upon heating.

When a general melanoidin structure is assumed, being built up mainly from sugar degradation products, probably branched via amino compounds (*6*), an oxidative degradation can certainly induce several changes. Two oxidation agents were applied to nonsoluble standard glucose/glycine melanoidins: potassium permanganate and sodium periodate. $KMnO_4$ is a strong, but relatively nonselective oxidant when used in aqueous solutions. In the melanoidin structure, primary and secondary alcohols can be converted to carboxylic acids and ketones, respectively; double bonds can be converted in diols or become oxidatively cleaved. Furan type molecules are predominantly expected after heating. $NaIO_4$ is widely used for the oxidative cleavage of 1,2-diols to yield aldehydes. These diol structures are, among others, present in the sugar-containing side chains in the melanoidins.

Oxidation of nonsoluble glucose/glycine melanoidins with $KMnO_4$ yielded only soluble LMW yellow degradation fragments. After heating of this fraction, mostly pyrroles were formed, in addition to some pyridines, some furans, 2-methyl-2-cyclopenten-1-one, pyrazine, and benzoxazole. Aside from this, aliphatic carbonyl compounds, such as 2- and 3-heptanone, 2- and 3-octanone, and 2- and 5-nonanone were detected. Also benzaldehyde, benzonitrile, and acetophenone were formed; their presence indicates an aromatization process induced by the oxidative treatment. Benzylic oxidations and oxidations of aromatic rings are known actions of $KMnO_4$.

Oxidation with $NaIO_4$ yielded mainly a nonsoluble fraction, consisting of 70 wt % of the original melanoidin, from which, upon thermal degradation, very few volatiles were formed: mainly furans, some pyrazines, and 2-cyclopentene-1,4-dione. After heating of the dried filtrate, some alkyl and aryl iodides were produced, as detected by GC/MS.

ABBREVIATIONS USED

COST: European Cooperation in the field of Scientific and Technical Research; HMW: high molecular weight; LMW: low molecular weight; N: nonsoluble; Sol: soluble; Glc: D-glucose; Gly: glycine; Glu: glutamic acid; ASA: L-(+)-ascorbic acid.

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Supporting Information Available: Table 3, listing separate GC-peak areas of the produced volatiles is available free of charge via the Internet at http://pubs.acs.org.

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