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Thermal Degradation Studies of Food Melanoidins

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Food melanoidins were isolated from bread crust, coffee, and tomato sauce and their composition was investigated by thermal degradation. Among the generated volatiles, important food flavor compounds were detected: in particular furans, carbonyl compounds, 1,3-dioxolanes, pyrroles, pyrazines, pyridines, thiophenes, and phenols. The results indicated that the isolated melanoidin fractions mainly consisted of compounds formed from carbohydrates and their degradation products. Besides proteins, other food constituents were incorporated in the melanoidin structure as well, such as lipid oxidation products in tomato melanoidins and phenolic compounds in coffee melanoidins. A comparison of the thermal generation of volatiles between these food-derived melanoidins and model melanoidins prepared from a single carbonyl compound and an amino acid showed that the degradation pattern of food melanoidins is quite different from that obtained from a glucose−glycine model system.

KEYWORDS: Maillard reaction; food melanoidins; thermal degradation

INTRODUCTION

During the cooking, processing, and storage of food products, a whole range of browning reactions occurs, initiated by the reaction of a carbohydrate with a compound possessing a free amino group. This complex network of reactions is known as the Maillard reaction and results in a wide variety of Maillard reaction products (MRPs). Due to the high reactivity of the intermediates, a complex polymerization takes place, resulting in brown-colored high molecular weight melanoidins. Thus, melanoidins comprise a substantial proportion of several food products, such as coffee, roasted malt, breakfast cereals, and bread, and are widely consumed dietary components. In addition, melanoidins possess antioxidant activity (*1*), are responsible for the development of color in heat-processed food products (*2*), may contribute to food texture, and are likely to play a role in the binding of nutritionally important metals (*3*), potentially undesirable dietary compounds (*4*), and flavor compounds (*5*). Still, the structures and characteristics of melanoidins remain poorly defined. Research has been devoted to melanoidins prepared from model reactions of a single amino acid and a carbohydrate. Different hypotheses have been formulated on the structural backbone of these model melanoidins. A first hypothesis states that a melanoidin skeleton is constituted mainly from sugar degradation products, polymerized through aldol-type condensations and probably branched via amino compounds (*6*−*8*). It was shown that, under water-free Maillard reaction conditions, significant amounts of carbohydrates are incorporated as side chains, with an intact glycosidic bond, into the melanoidin skeleton (*9*). Tressl et al. (*10*) proposed a complex macromolecular structure consisting of repeating units of furans and pyrroles, linked by polycondensation reactions. Hofmann (*11*) identified low-molecular weight chromophores and postulated the generation of melanoidin-type colorants by a cross-linking reaction between these low molecular weight substances and noncolored high molecular weight biopolymers, such as proteins.

It is generally accepted that variation of the substrates and of the reaction conditions strongly affect the structure of the resulting brown polymer (*8*, *12*). It can therefore be assumed that, in real food systems, different structures coexist.

One of the strategies in the search for melanoidin characterization is the chemical or thermal degradation of melanoidins (*6*, *7*), followed by the identification of the decomposition products formed. Although thermal degradation does not yield intact molecules that can be considered building blocks of the original macromolecule, it yields diagnostic products, giving information on structural domains of the melanoidin network. In addition, thermal destruction of melanoidins leads to the formation of volatiles that contribute to the development of aroma in roasted or baked food systems (*13*). Half of the volatile destruction products of beer melanoidins were known as beer flavor compounds, indicating the participation of melanoidins in the flavor formation process (*14*).

In previous research, the volatiles produced upon heating of standard model melanoidins were studied, which allowed a comparison between melanoidins prepared from glucose or

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ascorbic acid with glycine or glutamic acid (*15*, *16*). However, the general validity of the conclusions drawn from model systems and the relevance for complex real food systems remains unknown. Therefore, melanoidins were prepared from three different food systems in the present study, namely, bread crust, tomato puree, and coffee, and the different molecular weight fractions obtained were characterized by thermal degradation. The results allow a comparison of the thermal degradation pattern of three different food melanoidins with the results obtained from the thermal degradation of model melanoidins prepared from a single carbonyl compound with a single amino acid. In addition, food flavor compounds resulting from the thermal degradation of these food melanoidins are identified.

MATERIALS AND METHODS

Materials. All solvents and reagents used for the melanoidin preparations were of analytical grade and were supplied by Fluka. The following compounds were used as reference compounds for identification: benzaldehyde, 2,3-pentanedione, 2-methylfuran, 2,5-dimethylfuran, 2-acetylfuran, furfural, 2-furylmethanol, pyrazine, 2,5-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethyl-3,5(6)-dimethylpyrazine, 1,2,5-trimethyl-1*H*-pyrrole, 2-acetyl-1-methyl-1*H*-pyrrole, 1-methyl-1*H*-pyrrole-2-carbaldehyde, and styrene (Acros Organics, Geel, Belgium); 5-methylfurfural, 2-acetyl-5-methylfuran, benzofuran, 2-methylbenzofuran, 2-methylpyrazine, 2,5-dimethyl-1*H*-pyrrole, and 3-ethylpyridine (Sigma−Aldrich, Bornem, Belgium); 2,3-dimethylpyrazine, 2,6-dimethylpyrazine, and tetramethylpyrazine (Janssen Chimica, Beerse, Belgium); and acetoxyacetone (Fluka, Bornem, Belgium).

Preparation of Gluten−Glucose and Bread Crust Melanoidins (*17*). Gluten was prepared by extensive washing under tap water of dough obtained from wheat flour containing 9.5% proteins (brand Barilla). Glucose (2.6 g) was added to the gluten (13 g) and mixed. The mixture was heated in an oven (Memmert) at 150 °C for 45 min. The resulting brown cake was freeze-dried and ground in a blender. From 13 g of gluten (wet) and 2.6 g of glucose, 5.4 g of sample were obtained. Bread crust was separated with a kitchen knife from a 1-kg bread, type San Sebastiano, which is characterized by a thick and dark crust. The crust samples were freeze-dried and ground in a kitchen blender.

The melanoidins from bread crust and gluten−glucose were extracted in an aqueous environment by an enzymatic digestion with pronase E, from *Streptomyces griseus*. To monitor the enzymatic digestion, 250-mg samples were dissolved in 3 mL of Tris-HCl buffer (20 mM, pH 8) in triplicate. One series of three samples served as the control, while to another series of three samples the enzyme was added. Pronase was added to the samples up to a concentration of 0.1 mg/mL. In a separate flask, aliquots of bread crust (or of the gluten−glucose reaction mixture) were treated for digestion in the same way. The samples were carefully mixed (vortex) and incubated at 37 °C while shaking (75 rpm).

The digestion was monitored by spectrophotometric measurements. For this purpose, samples were centrifuged (4000*g*/min, 4 °C, 10 min), after which 40 μL of supernatant was added to 1 mL of distilled water before the absorbance was measured at 360 and 420 nm after 24, 48, and 118 h. After this time, the samples were centrifuged and the supernatants collected. Precipitation with trichloroacetic acid (TCA) was performed by adding TCA to the samples up to a concentration of 9.5%, 13%, 17.5%, and 20%, followed by centrifugation.

To fractionate the resulting melanoidin solution, ultrafiltration was performed with a stirred ultrafiltration cell (Amicon). Thus, three fractions were obtained: a high molecular weight fraction (HMW > 30 000), an intermediate molecular weight fraction (30 000 > IMW > 3000), and a low molecular weight fraction (LMW < 3000).

Preparation of Tomato Melanoidins. In a round-bottom flask, 350 g of sieved tomatoes (passata, brand AnnaLisa) were placed in a preheated water bath and allowed to reflux with stirring for 8 h (or 40 h). The sample was allowed to cool and was extracted with 5 × 250 mL of dichloromethane. Upon centrifugation (4000*g*/min, 10 min, 4 °C), the brownish aqueous phase containing the water-soluble melanoidins was collected. The HMW melanoidins were separated by dialysis [Medicell International, molecular weight cutoff (MWCO) 12 000−14 000] during 3 days (4 °C, with stirring). The water was removed by lyophilization. Fifty grams of triple-concentrated tomato puree (Oro di Parma), dissolved in 200 mL of water, was treated in the same way as the heated tomato purees.

Preparation of Coffee Melanoidins. Standard roasted coffee (100 g) was grounded to a fine powder in a blender. A first solid−liquid extraction was carried out by adding 300 mL of hot water (75 °C) and stirring for 5 min. The resulting mixture was filtered and the residue on the filter was extracted again with 300 mL of hot water (75 °C). After filtration, both filtrates were combined and defatted by extraction with dichloromethane (2 × 200 mL). The melanoidin solution was concentrated by lyophilization and fractionated in three fractions by ultrafiltration: a HMW fraction (>30 000), an IMW fraction (30 000 > MW > 3000), and a LMW fraction (<3000).

Absorbances of melanoidin solutions of specific concentrations (cf. figures) were recorded on a UV−Vis 2100 (Shimadzu).

Solid-Phase Microextraction. Silanized 4-mL SPME vials (Supelco, Bellefonte, PA) 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 (±5 °C) during 10 min. After cooling of the vials to room temperature, the SPME fiber (DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was exposed to the headspace of the heated melanoidin during 5 min.

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 m × 0.25 mm i.d.; coating thickness 0.25 μm) was used. Working conditions were as follows: injector, 250 °C; transfer line to MSD, 250 °C; oven temperature, start 40 °C and 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), and 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

Bread Crust Melanoidins. Two types of melanoidins were obtained: melanoidins from a gluten−glucose model system and melanoidins from bread crust. The model system prepared from wheat gluten protein and glucose under dry reaction conditions was selected as an intermediate step in the evolution from a model system consisting of an amino acid and a sugar toward a real food system (*18*).

Solubilization of colored material from gluten−glucose and bread crust is not possible by solvent or water extraction. Therefore, water-soluble melanoidins were obtained from gluten−glucose and bread crust by an enzymatic extraction procedure, as was developed for the extraction of melanoidins from bakery products (*17*). Gluten−glucose Maillard reaction products, or bread crust samples, were incubated with pronase in an aqueous buffer solution. At different time intervals, the absorbance of the liquid phase was compared with control samples to monitor the progress of the solubilization of the bread crust material. The absorbance increased strongly during the first 2 days, but no further increase was noted when the samples were incubated longer. A substantial amount of the bread crust material remained unsolubilized.

The aqueous melanoidin extracts were subjected to a trichloroacetic acid- (TCA-) fractionated precipitation, but upon centrifugation of the 20% TCA solution, only a small pellet of protein was removed (50 mg of dry weight/g of bread crust).

Table 1. Yield before and after Dialysis of Melanoidins from Different Tomato Samples

	yield before dialysis (mg/mL)	yield HMW melanoidins (%)
tomato puree, 8 h reflux	33.1	13.9
tomato puree, 40 h reflux	67.5	6.1
tomato puree, triple concentrated	73.6	2.3

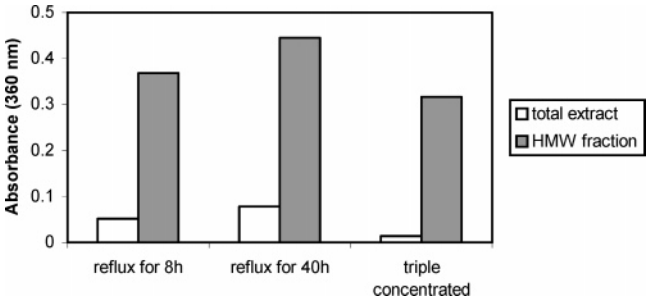


Figure 1. Absorbance at 360 nm of different melanoidin fractions isolated from different tomato puree samples (0.5 mg/mL).

This indicates that the enzymatic digestion procedure created mostly soluble small peptides. In the following experiments, the supernatants were fractionated immediately without TCA precipitation, to exclude later interference of acid remaining in the samples. Fractionation of bread crust melanoidins by ultrafiltration was preferred to gel filtration, since losses due to irreversible binding of browning products to the gel material have been reported (*19*).

Tomato Melanoidins. In a previous investigation of our research group, brown high molecular weight melanoidins with antioxidant activity were isolated from the water-soluble material of heated tomato purees (*20*). Elementary analysis showed a $C_9H_{14}NO_5$ composition, similar to that of melanoidins obtained from different carbohydrate−amino acid model systems (*12*). This is explained by the fact that the nitrogen-containing starting material of tomato (up to 1 g/100 g of fresh product) is mainly composed of free amino acids (*21*).

In this work, tomato melanoidins were prepared by different procedures. Canned tomatoes were subjected to a relatively mild heat treatment of 8 h at 90 °C and to a severe heat treatment of 40 h at 105 °C, both under reflux conditions. These described heat treatments are very severe as compared to common kitchen practice, although some recipes do require prolonged heating of tomato sauce. However, these procedures were necessary in order to collect sufficient melanoidin material. In addition, tomato melanoidins were obtained from a triple-concentrated tomato puree, by a similar procedure, but without additional heat treatment.

As is displayed in **Table 1**, only a very small fraction of the dry weight obtained before dialysis consisted of HMW melanoidins. In **Figure 1** the absorbance values (360 nm) of the different tomato melanoidin fractions are shown. This graph demonstrates that the HMW fractions showed a considerably higher absorbance than the total extracts before dialysis. A longer heating period of the tomato puree resulted in a darker colored solution, and the concentrated commercial tomato puree sample showed generally a lower absorbance than the heat-treated samples. A similar pattern was found by measuring the absorbance at 420 nm (data not shown).

These results confirm that in heated tomato the main contributors to the formation of water-soluble colored material are the low molecular weight compounds, such as free amino acids, glucose, and fructose. It has been shown that when melanoidins are formed in a glucose/protein model system, most of the colored material is present in the HMW fraction, while in heated glucose/amino acid solutions most of the colored compounds are of low molecular weight (*19*). In heated tomatoes about 90% of the colored material was eliminated during dialysis as LMW compounds. It should be noted, however, that the HMW fraction displays a visible absorption that is, at the same concentration, about 10-fold higher than that of the total extract.

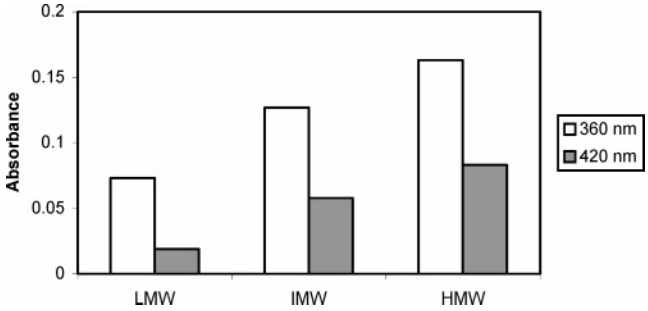


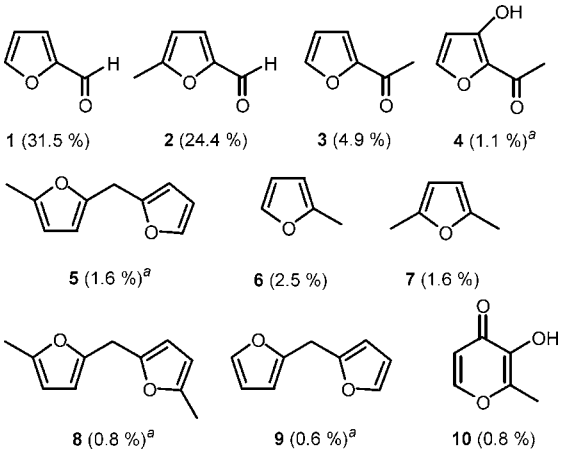
Figure 2. Absorbance at 360 and 420 nm of different melanoidin fractions of coffee (0.05 mg/mL).

Coffee Melanoidins. During coffee roasting, melanoidins are formed via the Maillard reaction, constituting about 23% of roasted coffee (*22*). In the present study, coffee melanoidins were isolated from roasted coffee beans according to a well-described protocol (*23*) and were fractionated by ultrafiltration into three different fractions: a high molecular weight fraction (HMW > 30 000), an intermediate molecular weight fraction (30 000 > IMW > 3000), and a low molecular weight fraction (LMW < 3000). The extracted colored material was composed of 25% HMW coffee melanoidins, 23% IMW coffee melanoidins, and 52% LMW coffee brew material. The absorbance of the different fractions, at the same concentration, is shown in **Figure 2**. The higher molecular weight fractions clearly displayed the darkest color and the highest UV absorbance, which is in agreement with previous studies (*17*). The brown color of the coffee melanoidins was considerably darker than for bread crust and tomato melanoidins.

Thermal Degradation Studies. Gluten−glucose melanoidins and the different molecular weight fractions obtained from bread crust melanoidins were heated at 250 °C (10 min), and the produced volatiles were analyzed by SPME−GC−MS. All analyses were performed in triplicate, except for LMW bread crust material, where very little product was available.

The headspace profile of the high molecular weight bread crust melanoidins consisted mainly of furans (74% of the total GC peak area). Various 2-alkyl-substituted 4,5-dimethyl-1,3-dioxolanes (14%) were detected, but the exact alkyl substituents could not be established for all compounds on the basis of mass spectrometry alone. Some selected substituted furans and maltol **10** that were generated from heated HMW bread crust melanoidins are depicted in **Chart 1**. The percentages displayed represent the share of the total GC peak area that was ascribed to a certain compound. Furfural **1**, maltol **10**, and isomaltol **4**, which are important compounds in the headspace of heated HMW bread crust melanoidins, are typical caramelization products of sugars. Methylene-bridged furan derivatives (**5**, **8**, and **9**) are indicators of glycosidically linked sugar residues. As opposed to these carbohydrate-derived flavor compounds, nitrogen-containing heterocycles were quantitatively of minor importance among the volatiles of HMW bread crust melanoidins.

Chart 1. Selected Volatiles Released after Heating (250 °C, 10 min) of HMW Bread Crust Melanoidins with the Percentage of the Total GC Peak Area



a Tentatively identified.

Heating of the IMW bread crust melanoidins yielded mostly the same compounds as the HMW fraction, but quantitatively more compounds were released from the IMW fraction (total GC peak area 8.2×10^9 as compared to 5.4×10^9 for the HMW fraction). In addition, heating resulted in 25% weight loss for the compounds of intermediate molecular weight, while 16% weight loss was found for HMW bread crust melanoidins.

In the case of the LMW fraction, 55% of the headspace profile consisted of pyrazines, in contrast with the low amounts of nitrogen-containing compounds detected after heating of the higher molecular weight fractions.

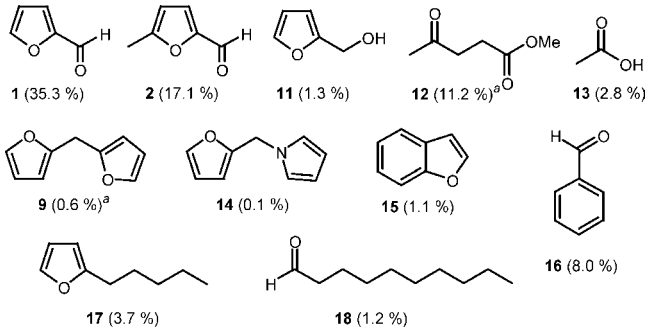
Application of an enzymatic digestion with pronase should result in the cleavage of most peptide bonds. Therefore, it can be expected that the HMW fraction mainly consists of carbohydrate-containing melanoidins, while the LMW fraction should be rich in small peptides and free amino acids, yielding mainly pyrazines upon heating. In fact, the higher molecular weight fractions yielded very few nitrogen-containing compounds upon heating. Thus, it can be concluded that very few nitrogen-containing compounds are incorporated in the bread crust melanoidin backbone with other than peptide bonds, for instance as pyrroles. Carbohydrates and their degradation products are most likely the main constituents of the melanoidin fractions isolated according to this procedure.

HMW melanoidins prepared from the gluten−glucose model system also generated mainly furans upon heating, in particular furfural **1** (41%) and 5-methylfurfural **2** (34%). No methylene-bridged furans were detected, but some pyrroles [1-(3-methylbutyl)-1*H*-pyrrole, 1-(3-methylbutyl)-1*H*-pyrrole-2-carbaldehyde, and 1-furfuryl-1*H*-pyrrole-2-carbaldehyde] and pyrazines (methylpyrazine, 2,3-dimethylpyrazine, and 3-ethyl-2,5-dimethylpyrazine) were formed.

Since free sugars (glucose and fructose) and free amino acids are important constituents of tomato dry matter (*24*), it can be expected that the formation of Maillard reaction products from tomato will show similarities with model melanoidins, prepared from a single sugar and amino acid, in particular with glucose−glutamic acid melanoidins.

Analysis of the volatiles released upon thermal degradation of tomato melanoidins revealed the presence of mostly furans (70−80% of the headspace profile) (**Chart 2**). The remaining of the headspace profile consisted mainly of carbonyl compounds, a small amount (1−2%) of pyrroles, and methyl

Chart 2. Main Volatiles Produced after Heating (250 °C, 10 min) of HMW Melanoidins Prepared from Tomato (Refluxed for 8 h) with the Percentage of the Total GC Peak Area



a Tentatively identified.

thiophene-2-carboxylate. From the concentrated (nonheated) tomato paste, 2-methylthiophene, and methylpyrazine were also generated. In general, the three tomato melanoidin preparations yielded very similar spectra of volatiles upon heating at 250 °C. The amount of volatiles released from the tomato melanoidins (as measured by the total GC peak area) increased with browning: concentrated tomato puree (4.6×10^9), tomatoes heated for 8 h (6.0×10^9), and tomatoes heated for 40 h (6.2×10^9). After heating, the three differently prepared tomato melanoidins showed a very similar weight loss of 25%.

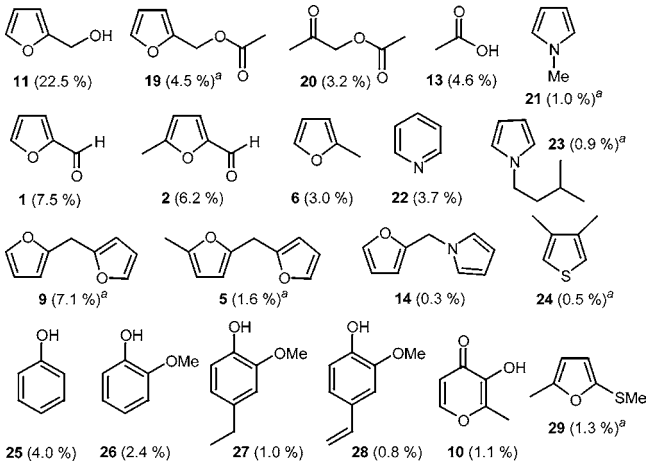
2-Pentylfuran **17**, generated by heating HMW tomato melanoidins, is an autoxidation product of linoleic acid (*25*). Aliphatic aldehydes detected, such as hexanal, heptanal, nonanal, and decanal **18**, are also typical lipid oxidation products. In the preparation of the tomato melanoidins, dichloromethane extraction has been performed in order to remove the fatty fraction, but some lipids may have been incorporated in the melanoidin skeleton. The formation of melanoidin-like colored polymers from the reaction of proteins with lipid oxidation products has been reported in the literature (*26*).

The results were compared with thermal degradation experiments of pectin (poly-D-α-galacturonic acid, commercial, from apple), since tomato pectin may have been isolated together with the melanoidins, according to the procedure described. Heating of pectin released mainly furfural **1** (48%), methylfuran-2-carboxylate (21%), and 5-methylfurfural **2** (15%). Some compounds detected in the headspace of heated tomato melanoidins may therefore result from pectin-like fractions, but most flavor compounds cannot be ascribed to tomato pectin.

It can be concluded that, during the preparation of tomato sauce, a relatively low amount of HMW light-colored polymers is formed through the reaction of sugars and amino acids, but with fatty compounds and pectin-like fractions taking part in the reaction. Therefore, in a complex food system, many food constituents can be included in the formation of the polymeric network, catalyzed by Maillard-type reactions.

Heating at 250 °C of coffee melanoidins and lower molecular weight compounds resulted in a weight loss of 16% for the HMW melanoidins, of 23% for the IMW fraction, and of 7% for the LMW compounds. Heating of HMW coffee melanoidins yielded a large variety of volatiles, among which furan compounds dominated. The lower molecular weight fractions yielded a larger amount of nitrogen-containing compounds, especially pyridines. Sulfur-containing compounds were also found in the headspace of heated coffee melanoidins, for example, 3,4-dimethylthiophene **24** and 2-methyl-5-methylthiofuran **29**.

Chart 3. Main Volatiles Produced after Heating (250 °C, 10 min) of HMW Coffee Melanoidins with the Percentage of the Total GC Peak Area



[a] Tentatively identified.

A selection of produced volatiles is depicted in **Chart 3**. The headspace profiles of the heated IMW and HMW coffee melanoidins were quite similar.

Most of the carbohydrates in coffee are insoluble polysaccharides, while monosaccharides hardly occur. Therefore, the reactive carbohydrate fraction in coffee is not very high, and typical sugar caramelization products such as furan-2-carbaldehydes do not predominate among the volatiles of heated coffee melanoidins, as was the case for bread crust and tomato melanoidins. Maltol **10**, which can be formed from disaccharides or Amadori compounds (*27*), has been identified in roasted coffee aroma and was detected among the volatiles of heated coffee melanoidins.

Sulfur-containing furan-type compounds, such as 2-[(methyldithio)methyl]furan, play an important role in the flavor of roasted coffee. 2-Furfurylthiol, the best-known sulfur-containing coffee flavor compound, was not detected upon heating of coffee melanoidin fractions. Other sulfur compounds produced, such as dimethyl disulfide and thiophenes (e.g., **24**), may result from the degradation of incorporated sulfur-containing amino acid residues.

Pyridines and pyrroles can be formed from the thermal decomposition of amino acids and from the interaction of amino acids with sugars or aliphatic aldehydes. Pyridine **22** and 3-methylpyridine, however, are also known degradation products of trigonelline (*N*-methylnicotinic acid), a known coffee constituent.

Essential constituents of coffee flavor are phenols, such as guaiacol **26**, 4-ethylguaiacol **27**, and 4-vinylguaiacol **28**. They are formed by the decarboxylation of phenolic carboxylic acids, which are apparently incorporated in the melanoidin structure. The presence of significant amounts of phenolic compounds in coffee melanoidins has been shown before (*23*). Among the degradation products resulting from Curie point pyrolysis (600 °C) of coffee melanoidins, one-third of the identified products were phenols (*28*). However, at these high temperatures, aromatization is induced and only a few compounds could be identified as chlorogenic acid degradation products.

The detection of a whole range of compounds, many resulting not only from carbohydrate−amino acid interactions, indicate that other coffee constituents, such as chlorogenic acids and trigonellin, are also involved in the browning reactions and are incorporated in the HMW structures formed.

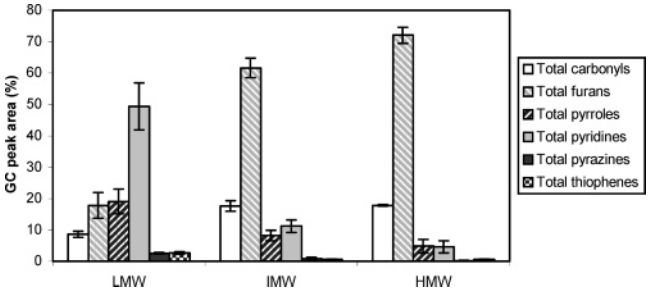


Figure 3. Thermal degradation profiles of different coffee melanoidin fractions (250 °C, 10 min).

The LMW fraction of the coffee brew yielded considerably more nitrogen-containing compounds upon heating as compared to the higher molecular fractions. Pyridine **22** accounted for 28% of the headspace profile. In **Figure 3**, a comparison is made of the share of the different functional groups represented in the headspace of heated coffee melanoidin fractions. This graph shows that the lower molecular fractions yielded especially nitrogen-containing heterocycles, while from the higher molecular fractions, considerably more furans and carbonyl compounds were released.

From these results it can be concluded that carbohydrate degradation products possess the highest polymerization capacity. Amino acids catalyze the conversion of carbohydrates to reactive degradation products, but seem to be incorporated to a lesser extent in the higher molecular weight melanoidin structures.

Comparison of Different Model and Food Melanoidins. In previous studies by our research group, various model melanoidin fractions have been subjected to thermal degradation, that is, glucose−glycine, glucose−glutamic acid, and ascorbic acid−glycine melanoidins (*15*). These results can be compared with the results obtained in the present study from glucose−gluten melanoidins as well as from melanoidins isolated from bread crust, tomato, and coffee. For each type of melanoidins and for each molecular weight fraction, a whole range of compounds was released upon heating, comprising on one hand quite universal and on the other hand very specific volatiles. A comparison can be made between the relative importance of the different chemical classes of compounds in the headspace profile of the different melanoidins. In **Table 2**, the relative shares of the total GC peak area of carbonyl compounds, furans, pyrroles, pyridines, pyrazines, 1,3-dioxolanes, thiophenes, phenols, and oxazoles are presented for 14 different melanoidin fractions. These data were subjected to principal component analysis (PCA) to get an insight in the variability of the data. The IMW fraction of bread and coffee melanoidins has been omitted for the construction of the graph for reasons of clarity and because of the high similarity with the HMW fraction. The PCA biplot is shown in **Figure 4**, depicting all melanoidin fractions in the plane of the two first principal components. Melanoidins with a high negative value of component 1 (explaining 31.5% of variance) released high amounts of furans, 1,3-dioxolanes, and carbonyl compounds upon heating and low amounts of especially pyridines, thiophenes, and phenols. Melanoidins with a high positive value of component 2 (explaining 27.5% of variance) yielded especially high amounts of furans, phenols, and thiophenes and low amounts of pyrroles and pyrazines. In **Figure 4** it can be seen that the melanoidin fractions derived from bread and tomato are not differentiated from each other on the basis of the flavor generation profile and are located on the left-hand upper side of the graph, indicating the generation of mainly furan and 1,3-dioxolane

Table 2. Volatiles Generated upon Heating of Different Melanoidin Fractions[a] Classified in Different Chemical Groups

	carbonyls	furans	pyrroles	pyridines	pyrazines	dioxolanes	thiophenes	phenols	oxazoles
GlutGlc_HMW	6.56	89.15	1.06	*b*	2.19	0.3	*b*	*b*	*b*
bread_LMW	*b*	17.77	7.38	2.29	55.04	*b*	*b*	*b*	*b*
bread_IMW	1.91	80.87	*b*	*b*	0.3	16.14	*b*	*b*	*b*
bread_HMW	2.68	73.84	0.23	*b*	8.37	14.41	*b*	*b*	*b*
coffee_LMW	4.77	16.16	17.7	47.02	2.38	*b*	2.52	5.78	*b*
coffee_IMW	14.98	51.68	7.01	9.48	0.74	*b*	0.46	12.34	*b*
coffee_HMW	16.01	64.55	4.48	4.02	0.19	*b*	0.5	8.17	*b*
tomato1_HMW	29.53	66.78	1.38	*b*	*b*	*b*	0.5	0.1	*b*
tomato2_HMW	19.05	77.46	1.63	*b*	*b*	*b*	0.22	0.55	*b*
tomato3_HMW	16.31	79.95	2.22	*b*	0.18	*b*	0.45	*b*	*b*
GlcGly_HMW	18.1	54.85	24.55	1.63	0.64	*b*	*b*	*b*	0.15
GlcGly_LMW	9.98	22.36	54.10	1.58	11.42	*b*	*b*	*b*	0.36
GlcGlu_HMW	8.17	91.83	*b*	*b*	*b*	*b*	*b*	*b*	*b*
ASAGly_HMW	20.87	33.92	41.89	0.72	1.84	*b*	*b*	*b*	0.13

[a] Quantities are expressed as percentage of total GC peak area. LMW = low molecular weight; IMW = intermediate molecular weight; HMW = high molecular weight; GlutGlc = gluten–glucose; GlcGly = glucose–glycine; GlcGlu = glucose–glutamic acid; ASAGly = ascorbic acid–glycine; tomato 1 = tomato paste, reflux 8 h; tomato 2 = tomato paste, reflux 40 h; tomato 3 = triple-concentrated tomato paste. [b] Not detected.

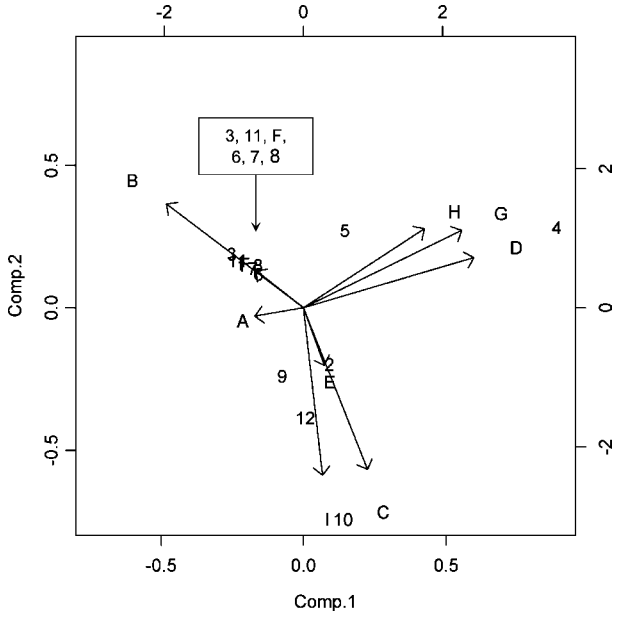


Figure 4. PCA biplot, depicting the different melanoidin fractions in the plain of the two first principal components (explaining 59% of variance). (1) Glucose–gluten HMW; (2) bread crust LMW; (3) bread crust HMW; (4) coffee LMW; (5) coffee HMW; (6) tomato, reflux 8 h, HMW; (7) tomato, reflux 40 h, HMW; (8) tomato, triple concentrated; (9) glucose–glycine LMW; (10) glucose–glycine HMW; (11) glucose–glutamic acid HMW; (12) ascorbic acid–glycine HMW; (A) carbonyl compounds; (B) furans; (C) pyrroles; (D) pyridines; (E) pyrazines; (F) 1,3-dioxolanes; (G) thiophenes; (H) phenols; (I) oxazoles.

compounds upon heating. Coffee melanoidins are differentiated from the others by the generation of phenolic compounds, pyridines, and thiophenes upon heating. The LMW fractions (bread, coffee) are clearly differentiated from the HMW melanoidins. Model melanoidins prepared from either glucose or ascorbic acid with glycine are differentiated from food-derived melanoidins by the yields of pyrroles, pyrazines, and oxazoles. As determined by the volatiles produced upon heating, the variability between glucose−glycine model melanoidins and food-derived melanoidins (bread, tomato) is high. Model melanoidins prepared from glucose and glutamic acid showed a much higher similarity to food-derived melanoidins, based on thermal degradation patterns. There is an almost complete overlap of glucose−glutamic acid, glucose−gluten, bread crust, and three types of tomato HMW melanoidins. The low reactivity of glutamic acid in the Maillard reaction, as compared to the highly reactive glycine, results in melanoidins with little incorporation of amino acid degradation products. The amino acid probably mainly catalyzes the conversion of the sugar in reactive degradation products that are able to polymerize. This situation seems to describe more adequately what happens in real food systems, indicating that glucose−glycine melanoidins are perhaps not the best model system to study the properties of food-derived melanoidins.

ABBREVIATIONS USED

HMW, high molecular weight; IMW, intermediate molecular weight; LMW, low molecular weight; PCA, principal components analysis; TCA, trichloroacetic acid.

Supporting Information Available: Three tables containing all volatiles, with corresponding retention index and GC peak area percentage, for different melanoidin fractions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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