JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Thermal Degradation Studies of Glucose/Glycine Melanoidins

KOUROSCH ABBASPOUR TEHRANI,[†,‡] MILDA KERSIENE,[†,§] AN ADAMS,[†] RIMANTAS VENSKUTONIS,[§] AND NORBERT DE KIMPE*,[†]

Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium, and Department of Food Technology, Kaunas University of Technology, Radvilenu pl. 19, Kaunas LT-3028, Lithuania

Nondialyzable and water-insoluble melanoidins, isolated from a glucose/glycine model reaction mixture, which was prepared in a standardized way according to the guidelines of the COST Action 919, were heated at different temperatures ranging from 100 to 300 °C. Among the volatile compounds, which were analyzed by SPME and GC-MS, pyrazines, pyridines, pyrroles, and furans were detected. In general, total amounts of volatile compounds increased with the temperature. When water-insoluble melanoidins were heated, especially at higher temperatures, this resulted in a higher diversity of isolated compounds. For furans, pyrroles, pyrazines, and carbonyl compounds a maximum was observed in the case of high molecular weight melanoidins around 200−220 °C. Pyridines and total oxazoles, however, were generated in higher yields with increasing temperatures. These results demonstrate the possibility of producing some flavor-significant volatiles from heated standard melanoidins at temperatures relevant to food preparation and contribute to the flavor aspects originating from melanoidins.

KEYWORDS: Maillard reaction; melanoidins; degradation; pyrolysis; glucose; glycine; flavors

INTRODUCTION

The Maillard reaction, the reaction between reducing sugars and compounds possessing a free amino group, is important for food processing, as it leads to the development of substances that affect color and flavor. The colored products of the Maillard reaction can be divided in two main groups: (1) the low molecular weight compounds (MW < 1000) and (2) the macromolecules, also known as melanoidins. Until now, due to the complexity of the system, it has not been possible to characterize completely a melanoidin from foods. Therefore, most of the characterization experiments have focused on melanoidins isolated from browned model systems. Because direct spectroscopic analysis of complex melanoidin fractions is far too difficult, most efforts have focused on the easier, although still very complex, identification of low molecular weight intermediates as possible monomers or simpler structural units involved in melanoidin formation. On the other hand, it was thought that the chemical decomposition of certain melanoidins and the identification of the degradation products formed might provide extra information on certain structural entities incorporated in melanoidins. Studies have already been done in which a nondialyzable melanoidin fraction from glucose/*n*-butylamine and glucose/NH_3 mixtures was pyrolyzed at 300−600 °C. Among the volatile pyrolysates, 1-butylaziridines and three alkylpyrroles could be tentatively identified (*1*, *2*). Similarly, nondialyzable melanoidins prepared from glucose and glycine, upon heating, gave rise to furans, toluene, pyrroles, pyridines, and acetic acid (*3*). However these volatiles, being common Maillard reaction products, may have been formed by recombination of pyrolysis intermediates. From the flavor generation point of view, pyrolysis of melanoidins has not been studied systematically. Some papers report the use of model melanoidins as a source of thermally generated aroma, as exemplified by the better organoleptic properties of bread prepared from dough with extra-added melanoidins (*4*). Melanoidins can thus undergo thermal destruction to lower molecular weight products and volatiles, which then may take part in the aroma formation. In fact, during roasting or baking, Maillard reaction products (MRPs) are formed by solid-phase interactions, processes that can be mimicked through pyrolysis experiments (*5*). Therefore, pyrolytic methods and their results can be interesting for food chemists, for example, for the fast analysis of caramel, thickening agents, and brown polymers formed by the Maillard reaction (*6*).

Due to the complexity and multiplicity of the nonvolatile MRPs, extensive studies have been performed on melanoidins prepared from model reaction systems. The browning reaction between glycine and glucose was especially investigated because of the ubiquitous presence of glucose in foods and the structural simplicity of glycine (*7*−*9*). Many laboratories have prepared and studied melanoidins from glycine−glucose model systems.

* Author to whom correspondence should be addressed (telephone 00 32 9 264 59 51; fax 00 32 9 264 62 43; e-mail Norbert.Dekimpe@rug.ac.be.
† Ghent University.
‡ Postdoctoral Fellow of the F.W.O.−Flanders (Belgium). Present address: Department of Organic Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.
§ Kaunas University of Technology.

10.1021/jf0116247 CCC: $22.00 © 2002 American Chemical Society
Published on Web 05/29/2002

However, because various reactants and heating conditions have been used, this resulted in studies that are difficult to compare in a meaningful way. Therefore, a standardized protocol was developed as part of a European Research Programme (COST Action 919), in which a standard protocol was designed by the different partners for the preparation of melanoidins, starting from glucose and glycine (*10*).

To shed more light on the flavor generation profile in heated melanoidins, especially melanoidins made in a standardized way, both the nondialyzable fractions, that is the water-soluble, and the water-insoluble melanoidins of a glucose−glycine model system were decomposed thermally in this research, using simple laboratory equipment. The volatile compounds obtained by heating of the melanoidins at different temperatures were analyzed by GC-MS.

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. This cellulose membrane retains >90% cytochrome *c* (MW 12400) in solution over a 10 h period. The dialysis tubing was prepared according to the manufacturer's instructions.

Preparation of Melanoidins. Glucose (0.05 mol, 9.00 g) and glycine (0.05 mol, 3.75 g) were placed in a 300 mL Christ filter bottle and 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 of the water had been removed (i.e., to 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 at 4 °C 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, and the liquid obtained after washing was mixed with the original filtrate. This solution (solution A) was made up to 250 mL with distilled water in a volumetric flask. The residue obtained, the so-called water-insoluble nondialyzable fraction of the melanoidins, was frozen, freeze-dried, and stored at −32 °C until further use. This fraction will be further designated the water-insoluble melanoidins.

Isolation of Water-Soluble Nondialyzable Melanoidins by Dialysis. Fifty milliliters of 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 two changes of the surrounding water. The dialysates, containing the low molecular weight fraction (LMW) of melanoidins, were not collected. At the end of the dialysis the contents of the dialysis tubing with the high molecular weight fraction (HMW) or the so-called nondialyzable melanoidins, were transferred to a 500 mL round-bottom flask, frozen in a liquid nitrogen bath, and freeze-dried until all of the water had been removed. The HMW water-soluble melanoidins were stored in a desiccator in a freezer at −32 °C until further use.

SPME. Silanized 4-mL SPME vials (Supelco Inc., Bellefonte, PA) 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 (PDMS 100 μm, Supelco Inc.) was exposed to the headspace of the heated melanoidin during 5 min. In method A the melanoidin was heated again at a higher temperature, whereas in method B for every new heating experiment new melanoidins were used.

Mass Spectrometry. For the analysis of the SPME extracts a Hewlett-Packard 6890 GC Plus coupled with an HP 5973 mass selective detector (MSD, quadrupole type), equipped with a CIS-4 programmed temperature vaporization (PTV) injector (Gerstel), and an HP5-MS capillary column (30 × 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, hold 2 min, programmed from 40 to 150 °C at 5 °C min^{-1} and from 150 to 260 °C at 10 °C min^{-1}, hold 5 min; carrier gas (He), 1.2 mL min^{-1}; splitless; ionization EI, 70 eV. Acquisition parameters were as follows: scanned *m*/*z* 40−200 (0−2 min), 40−300 (2−15 min), 40−400 (>15 min). Substances were identified by comparison of their mass spectra and retention times with those of reference substances and by comparison with the NIST Mass Spectral Library (version 1.6d, 1998). When only MS data were available, identities were considered to be tentative.

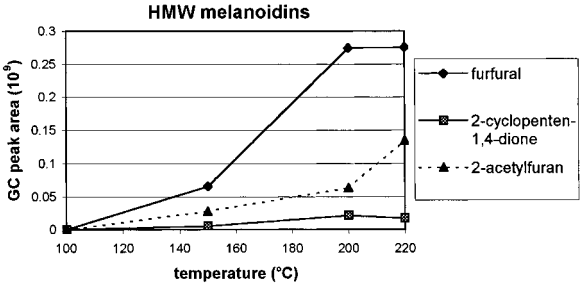

Figure 1. Influence of temperature on peak areas of selected furans and 2-cyclopentene-1,4-dione (method A).

RESULTS AND DISCUSSION

HMW melanoidins (water-soluble) were obtained from the model system D-glucose−glycine in a molar ratio of 1:1 in an anhydrous medium at 125 °C (see Materials and Methods). During the synthesis the reaction mixture lost ∼32% of its initial weight. Finally, 119 mg of nondialyzable melanoidins were obtained by dialysis along with 5 g of water-insoluble melanoidins.

First, the headspace of the freshly prepared melanoidins was analyzed at room temperature by means of SPME. The headspace of the HMW fraction revealed the presence of 2,6-dimethylpyridine and 1-methyl-2-pyrrolidinone during one week. This amount was only a fraction of the quantities found in the heated melanoidins and decreased with the time. The other identified compounds disappeared after 6 days; their starting levels constitute only a minor percentage of the volatiles detected in the heated HMW fraction. In the headspace of the water-insoluble melanoidins only three compounds were identified, which all disappeared after 6 days. Probably these residual compounds originate from the melanoidin preparation and are slowly released from the melanoidin matrix. Therefore, it can be expected that the initial temperature of 100 °C in the degradation experiments does enhance this flavor release process in the beginning together with a starting degradation of the melanoidin itself. 2-Pyrrolidinone and *N*-methyl-2-pyrrolidinone most likely originate from plasticizers.

Next, 50 mg of water-insoluble or HMW melanoidins was heated, using a sand bath at 100 °C. After cooling to room temperature, the headspace was sampled by means of SPME during 5 min and subsequently analyzed by GC-MS. After 1 h, the same sample was heated again on a sand bath of 150 °C followed by the same extraction procedure. This process was repeated also at 200 and 220 °C (method A). 5-Methyl-2-furancarboxaldehyde, furfural, and 2-acetylfuran were present both in the HMW and in the water-insoluble melanoidins headspace fraction, although in a 10-fold lower quantity (**Figures 1** and **2**). In addition, 2-acetyl-5-methylfuran was also formed in increasing amounts with increasing temperature of the water-insoluble melanoidins (**Figure 3**). Furfural and

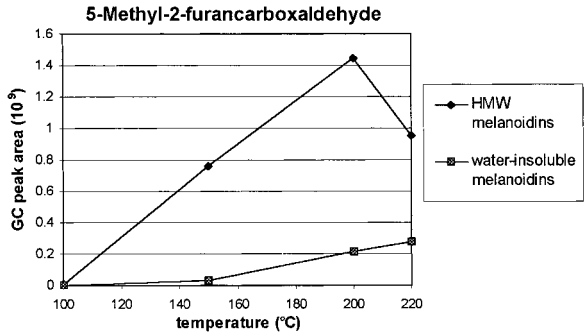

Figure 2. Comparison between formation of 5-methyl-2-furancarboxaldehyde in HMW and in water-insoluble melanoidins: influence of temperature (method A).

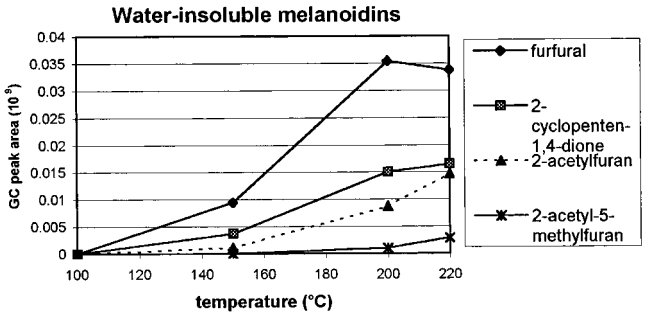

Figure 3. Influence of temperature on peak areas of selected furans and 2-cyclopentene-1,4-dione (method A).

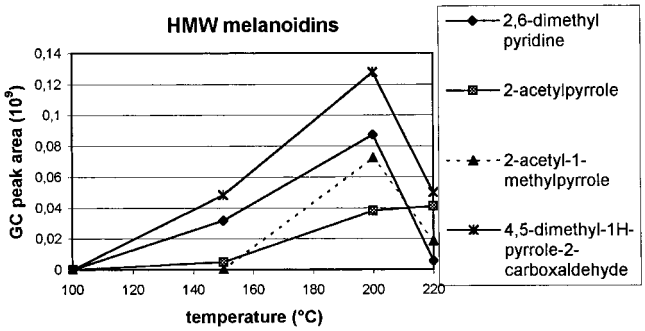

Figure 4. Influence of temperature on peak areas of pyrroles and pyridines (method A).

2-acetylfuran are typically present in the gas phase of heated glucose model systems (*11*). However, in our case the formation of furans can hardly be attributed to the residual presence of glucose in the melanoidin matrix because of the preparation protocol used (*12*), in which all of the eventually nonreacted glucose is washed away. Among the nitrogen-containing volatiles, 2-pyrrolidinone was the most abundant one, followed by 2-formylpyrrole. Also 4,5-dimethylpyrrole-2-carboxaldehyde, 2-acetyl-1-methylpyrrole, and 2-acetylpyrrole were identified (**Figures 4** and **5**). Again, these compounds were also formed in the water-insoluble melanoidins volatiles fraction, but in a 10-fold lower quantity (**Figure 6**). Two pyridines, that is, 2,6-dimethylpyridine and 2-acetylpyridine, were identified in the HMW (**Figure 4**) and the water-insoluble fraction, respectively (**Figure 6**). Pyrroles are generally less abundant in foods, and if formed, temperatures are, as illustrated here for the water-insoluble melanoidins, rather high (*13*). In the literature melanoidin-like oligomers have been prepared starting from *N*-substituted 2-formylpyrroles with *N*-methylpyrrole (*14*). The pyrroles observed in the degradation studies here might therefore constitute building blocks of the heated glucose−glycine

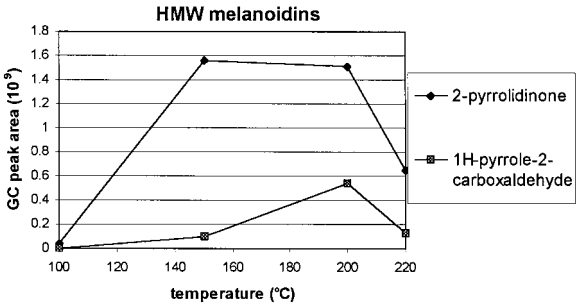

Figure 5. Influence of temperature on peak areas of 2-pyrrolidinone and 1*H*-pyrrole-2-carboxaldehyde (method A).

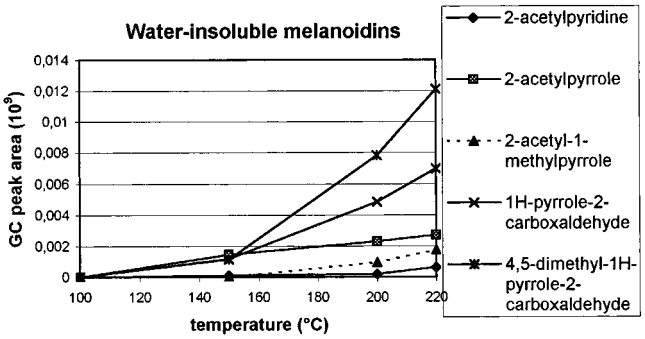

Figure 6. Influence of temperature on peak areas of pyrroles and pyridines (method A).

melanoidins. Kato and Tsuchida, however, suggested that pyrrole rings are not naturally present in native melanoidins but are formed by melanoidin pyrolysis (*1*). Of course, the higher temperatures (600 °C) used by this group and the fact that the melanoidins were prepared in an aqueous system hamper a good comparison between these results.

Among the different pyrazines formed, methylpyrazine is the most abundant one. By heating water-insoluble melanoidins levels of pyrazines, pyrroles, and furans increase with temperature, except for furfural. On the other hand, by heating the HMW fraction, the presence of 5-methyl-2-furancarboxaldehyde, furfural, pyrazines, 2,6-dimethylpyridine, and pyrroles shows a maximum at ∼200 °C.

In a second approach, freshly prepared amounts of melanoidins were heated at five different temperatures: 100, 150, 200, 250, and 300 °C (method B). For each experiment new melanoidins were used, so volatile levels in the headspace were generally higher than in the previous approach. Also, the generation of volatiles was more pronounced in the case of water-insoluble melanoidins (52 compounds identified) than with HMW melanoidins (35 compounds identified). In addition to the above-mentioned furans, also 2-furanylmethanol was detected in the heated HMW melanoidin system (**Figure 9**). Again, maximum levels of 5-methylfurfural were detected at 200 °C for the HMW melanoidins (**Figure 10**). Furans generally have very pleasant odors and largely determine the odor of processed foods. For example, furfural has a freshly baked bread odor, 5-methylfurfural a sweet caramel-like flavor, and 2-acetylfuran a sweet balsamic odor. Two cyclic ketones, 2-cyclopentenone and 2-cyclopentene-1,4-dione, were also detected (**Figures 9** and **11**). Oxazoles, such as 4,5-dimethyloxazole, benzoxazole, and 2,5-dimethylbenzoxazole, are mainly formed at higher temperatures. Alkylated oxazoles have been identified in various processed foods such as coffee, cocoa, roasted green tea, meat, and baked potato. Their mechanism of formation is not well investigated, although some propositions have been made in the literature (*15*). It is remarkable that no oxazoles

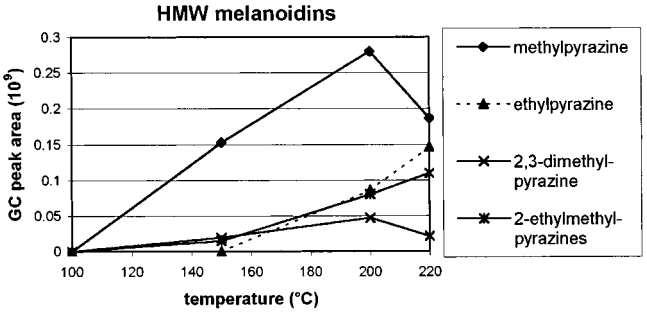

Figure 7. Influence of temperature on peak areas of pyrazines (method A).

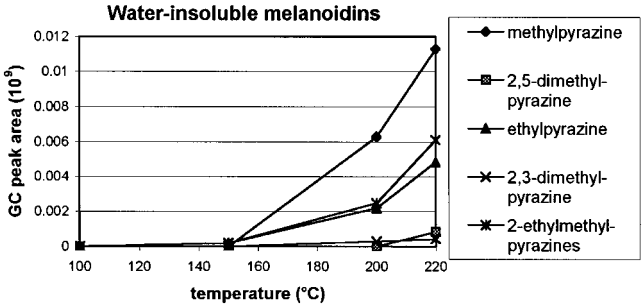

Figure 8. Influence of temperature on peak areas of pyrazines (method A).

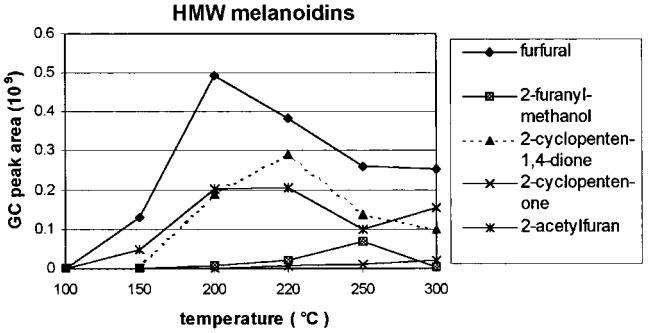

Figure 9. Influence of temperature on peak areas of furans, 2-cyclopenten-1-one, and 2-cyclopentene-1,4-dione (method B).

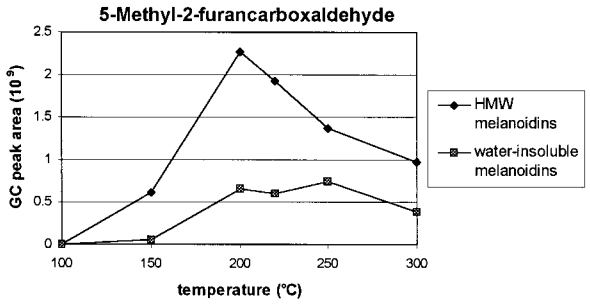

Figure 10. Comparison between formation of 5-methyl-2-furancarboxaldehyde in HMW and in water-insoluble melanoidins: influence of temperature (method B).

have been isolated when freeze-dried mixtures of glycine and glucose were heated during 1 h at 180 °C (*15*). The formation of 4,5-dimethyloxazole is explained by direct reaction of glycine with diacetyl, which originates from glucose. In our model system never a trace of diacetyl was detected. In another possible pathway 2-aminobutanone, formed by Strecker degradation of diacetyl and glycine, reacts with formaldehyde (*15*). Benzoxazole has been isolated as an oxidation product of lysozyme and glucose at 155 °C (*16*). Pyrroles only start forming at

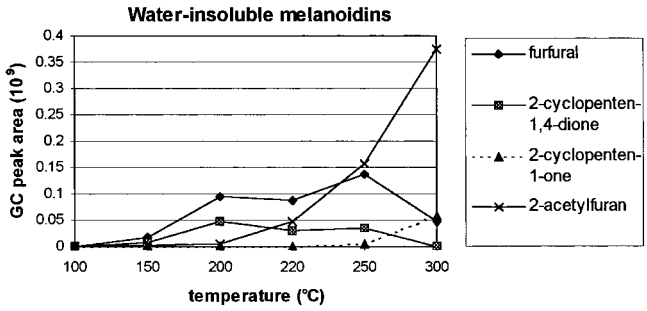

Figure 11. Influence of temperature on peak areas of furans, 2-cyclopenten-1-one, and 2-cyclopentene-1,4-dione (method B).

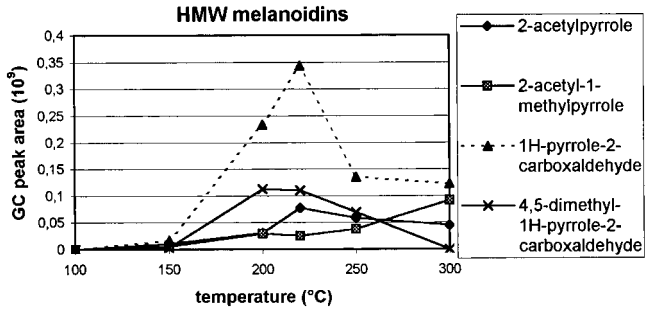

Figure 12. Influence of temperature on peak areas of pyrroles (method B).

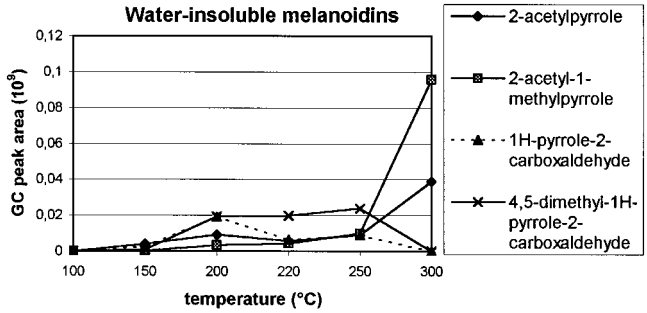

Figure 13. Influence of temperature on peak areas of pyrroles (method B).

temperatures of 150 °C, and by raising this temperature to 200−220 °C a maximum is reached. Both for the HMW and the water-insoluble melanoidin fraction 2-acetyl-1-methylpyrrole is formed in even higher amounts at 300 °C (**Figures 12** and **13**). Pyrroles have been reported in various heated foods, especially coffee. They seem not to be present in fresh, raw foods. Alkyl- and acylpyrroles generally have unfavorable odors, but upon dilution, alkylpyrroles exhibit a sweet, slightly burntlike aroma (*13*).

In a single case, that is, by heating water-insoluble melanoidin at 305 °C during 10 min, was 2-(2-furanylmethyl)-5-methylfuran identified. The isolation of such furanoid species could indicate the formation of glycosidically linked sugar derivatives (*17*). The latter fact is also supported by the high levels of the above-mentioned furaldehydes, which can be formed by thermally and electron-impact induced fragmentations of bridged structures between two or three furan nuclei (*18*). The mode of formation of difurylmethanes, however, is not yet clear (*19*). On the other hand, it is also possible that compounds such as 2-furanylmethanol, 5-methyl-2-furancarboxaldehyde, 2-acetylfuran, and 2-acetylpyrrole are formed from 3-deoxyglucosone as stated by Tressl et al. (*20*). Similar conclusions have been drawn in a study where melanoidins obtained from a glucose and glycine system in methanol were pyrolyzed at 350 °C (*10*). Upon

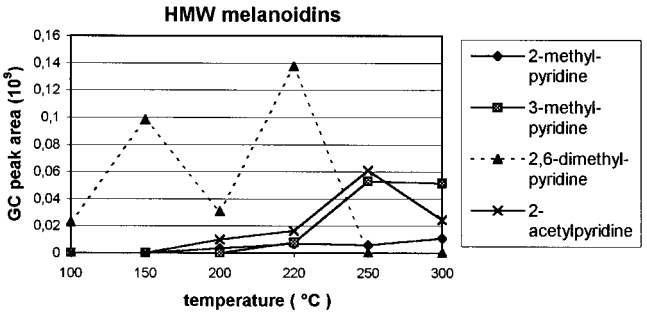

Figure 14. Influence of temperature on peak areas of pyridines (method B).

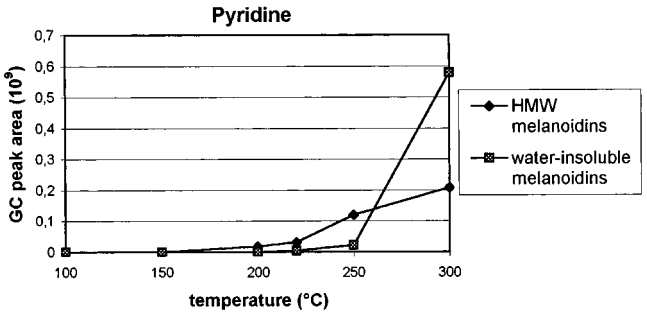

Figure 15. Comparison between the formation of pyridine in HMW and in water-insoluble melanoidins: influence of temperature (method B).

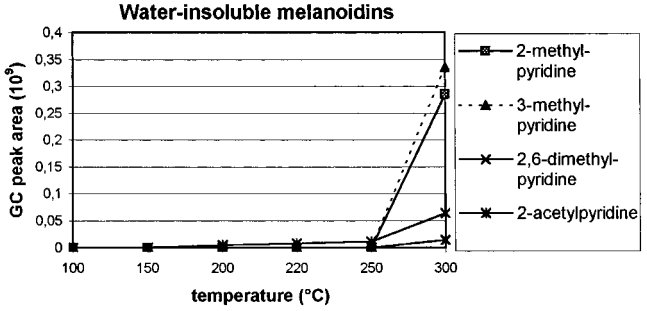

Figure 16. Influence of temperature on peak areas of pyridines (method B).

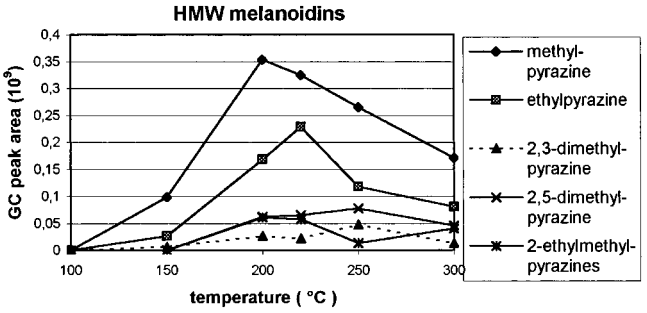

Figure 17. Influence of temperature on peak areas of pyrazines (method B).

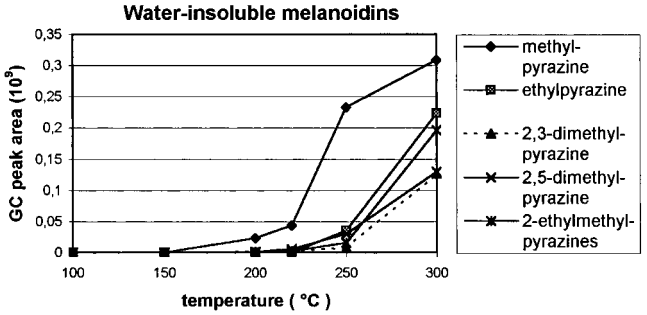

Figure 18. Influence of temperature on peak areas of pyrazines (method B).

thermal degradation 35 compounds were isolated, of which only 13 compounds were also found in our degradation studies. The fact that apparently analogous melanoidins yield so different results again proves the need to further investigate the properties of a standard melanoidin, as stated by the European COST Action 919 (*10*). *N*-Methylsuccinimide could be unambiguously detected at 200 and 220 °C when HMW melanoidins were heated. A 6-fold lower amount was detected when water-insoluble melanoidin was degraded at 250 °C. *N*-Methylsuccinimide is reported in the literature as a pyrolysis product of polyglycine at 500 °C (*21*). It is possible that nonreacted glycine is present in the melanoidins as a water-insoluble polypeptide. Analogous to the pyrolysis of glucose and glycine, trimethylpyrazine was only detected starting from 250 °C in the headspace of HMW melanoidins (*22*). As can be inferred from **Figure 14** the formation of 2,6-dimethylpyridine is quite irregular. Because this pyridine has never been detected before in Maillard mixtures, it probably originates from sources other than thermal degradation. The other pyridines, however, are frequently found in model reactions (*15*). From **Figures 15** and **16** it can be seen that pyridines are present in higher levels at higher temperatures in the case of the water-insoluble melanoidins. The generation of pyridine, however, is almost 3 times higher when HMW melanoidins are heated at 300 °C (**Figure 15**). Pyridine was the first pyridine found in a browning model system (*23*). It is worth mentioning that in the case of the water-insoluble melanoidins 12 different pyridines were identified, whereas only 5 pyridines were found for the HMW melanoidins. Pyridines have been reported in coffee, barley, roasted lamb, and meat. Some pyridines possess pleasant odors; however, most pyridines have green, bitter, astringent, roasted, burnt, pungent vegetable, or phenolic properties. In general, alkylpyridines possess a less desirable odor, whereas acylpyridines have more pleasant aromas. 2-Acetylpyridine has a cracker-type aroma (*15*).

Twelve pyrazines were detected in water-insoluble melanoidins, of which 10 also are formed in the heated HMW melanoidins. Although the water-insoluble melanoidins show higher yields of pyrazines at the higher temperatures, the HMW-derived pyrazines are formed in maximum quantities at ~200 °C (**Figures 17** and **18**). It is known that levels of pyrazines in Maillard reactions increase with temperature (*24*). Under all circumstances methylpyrazine and ethylpyrazine were the most abundant. Among the *N*-heterocycles, the pyrazines exhibit the most agreeable odors. Alkylpyrazines are mostly associated with heated food systems; they have a roasted nutlike flavor. Especially α-acetyl-*N*-heterocycles have a typical roast flavor character, with extremely low odor thresholds.

Finally, 2 g of water-insoluble melanoidins was heated on a sand bath at 260 °C, and the volatiles were trapped by means of liquid nitrogen during 30 min. The condensed compounds were then taken up in ether and after drying of this solution with magnesium sulfate and filtering off the drying agent; the filtrate was analyzed by means of GC-MS. Of 39 identified compounds, 9 were not detected in the other pyrolysates. Most of them are polymethylated pyrroles, which also have been isolated by other groups (*1*, *2*). *N*-Methylacetamide, detected under our circumstances, has been found also by pyrolysis of polyglycine (*20*).

In conclusion, total amounts of flavor compounds increase with the temperature. When water-insoluble melanoidins were heated, especially at higher temperatures, this resulted in a higher

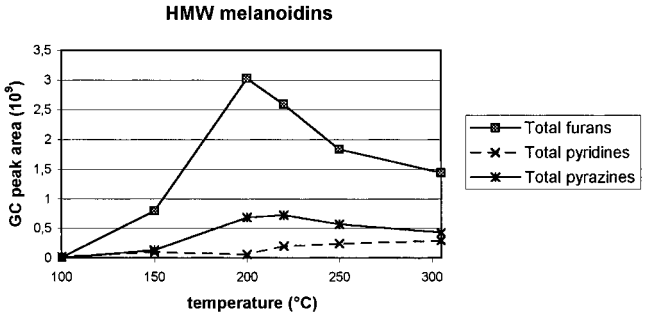

Figure 19. Influence of temperature on peak areas of total furans, pyridines, and pyrazines (method B).

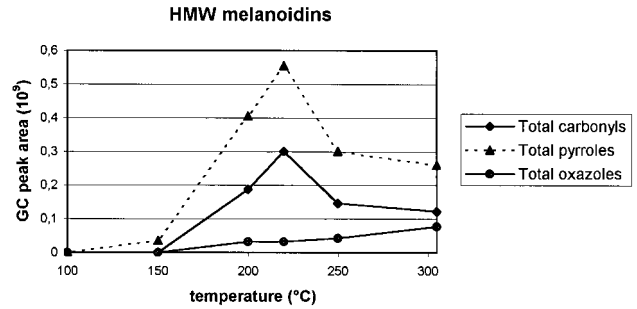

Figure 20. Influence of temperature on peak areas of total carbonyls, pyrroles, and oxazoles (method B).

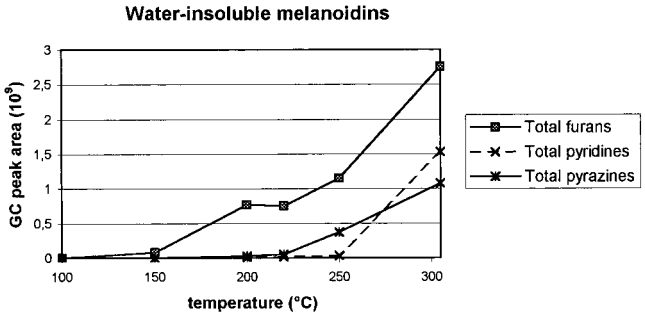

Figure 21. Influence of temperature on peak areas of total furans, pyridines, and pyrazines (method B).

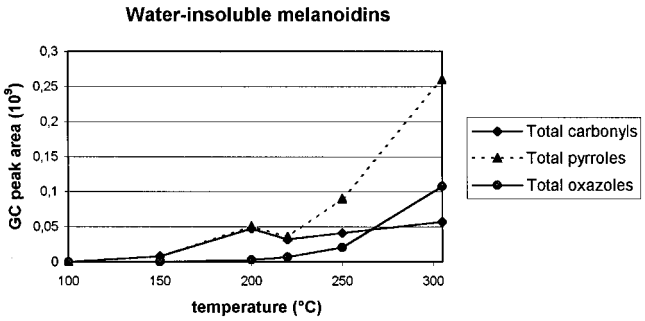

Figure 22. Influence of temperature on peak areas of total carbonyls, pyrroles, and oxazoles (method B).

diversity of isolated compounds. From **Figures 19** and **20** it can be seen that for furans, pyrroles, pyrazines, and carbonyl compounds a maximum was observed in the case of HMW melanoidins at ~200−220 °C. Pyridines and total oxazoles, however, continue to be generated in higher yields with increasing temperatures. For the water-insoluble melanoidin fractions all classes of compounds are formed in higher amounts with higher temperatures (**Figures 21** and **22**). Furans were in all cases the most abundant, followed by the pyrazines, carbonyl compounds, pyridines, pyrroles, and finally oxazoles. However, when looking at the individual compounds generated in the water-insoluble melanoidin system, we found amounts of furfural, 2-cyclopenten-1-one, 5-methylfurfural, and 4,5-dimethyl-1*H*-pyrrole-2-carboxaldehyde to decrease starting from 250 °C. It also has to be stressed that classical pyrolysis uses much higher temperatures (up to 600 °C), thus converting initially formed fragments into related structures as exemplified by oxidation of 2-furanylmethanols to the corresponding aldehydes and subsequently to the furanyl carboxylic acids (*16*). Therefore, by working at moderate temperatures, which resemble real food preparation conditions, more relevant results can be obtained.

Supporting Information Available: Tables 1−5, listing GC peak areas of the produced volatiles. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Kato, H.; Tsuchida, H. Estimation of melanoidin structure by pyrolysis and oxidation. *Prog. Food Nutr. Sci*. **1981**, *5*, 147−156.

(2) Tsuchida, H.; Komoto, M.; Kato, H.; Kurata, T.; Fujimaki, M. Melanoidin produced from the reaction of glucose and ammonia. Part V. Identification of heterocyclic nitrogen compounds produced by pyrolysis of the nondialyzable melanoidins. *Agric. Biol. Chem.* **1976**, *40*, 2051−2056.

(3) Hayase, F.; Kato, H. Volatile compounds formed by thermal degradation of nondialyzable melanoidins prepared from sugar-amino acid reaction systems. *Agric. Biol. Chem.* **1981**, *45*, 2559−2567.

(4) Kuncheva, M.; Rogacheva, S.; Obretenov, T. Model melanoidins as a source of thermally generated aroma. *Nauchni Tr.−Vissh Inst. Khranit. Vkusova Promst., Plovdiv* **1998**, *43*, 427−430.

(5) Baltes, W.; Mevissen, L. Model reactions on roast aroma formation. *Z. Lebensm. Unters. Forsch.* **1988**, *214*, 187−209.

(6) Baltes, W. Application of pyrolytic methods in food chemistry. *J. Anal. Appl. Pyrolysis* **1986**, *8*, 533−545.

(7) Homa, S.; Tomura, T.; Fujimaki, M. Fractionation of non-dialyzable melanoidin into components by electrofocusing electrophoresis. *Agric. Biol. Chem.* **1982**, *46*, 1791−1796.

(8) Kato, H.; Matsuda, T.; Kato, N.; Watanabe, K.; Nakamura, R. Browning and insolubilization of ovalbumin by the Maillard reaction with some aldohexoses. *J. Agric. Food Chem.* **1986**, *34*, 351−355.

(9) Wedzicha, B. L.; Kaputo, M. T. Reaction of melanoidins from glucose and glycine: composition, characteristics and reactivity towards sulphite ion. *Food Chem.* **1992**, *43*, 359−367.

(10) Ames, J. M.; Caemmerer, B.; Velisek, J.; Cejpek, K.; Obretenov, T.; Cioroi, M. The nature of melanoidins and their investigation. In *Melanoidins in Food and Health*; Ames, J. M., Ed.; 2000; Vol. 1, pp 13−29.

(11) Umano, K.; Hagi, Y.; Nakahara, K.; Shyoji, A.; Shibamoto, T. Volatile chemicals formed in the headspace of a heated D-glucose/L-cysteine Maillard Model System. *J. Agric. Food Chem.* **1995**, *43*, 2212−2218.

(12) Benzing-Purdie, L. M.; Ripmeester, J. A.; Racliffe, C. I. Effects of temperature on Maillard reaction products. *J. Agric. Food Chem.* **1985**, *33*, 31−33.

(13) Maga, J. A. Pyrroles in foods. *J. Agric. Food Chem.* **1981**, *29*, 691−694.

(14) Tressl, R.; Wondrak, G. T.; Garbe, L.-A.; Krüger, R.-P.; Rewicki, D. Pentoses and hexoses as sources of new melanoidin-like Maillard polymers. *J. Agric. Food. Chem.* **1998**, *46*, 1765−1776.

(15) Hwang, H.-I.; Hartman, T. G.; Ho, C.-T. Relative reactivities of amino acids in the formation of pyridines, pyrroles and oxazoles. *J. Agric. Food Chem.* **1995**, *43*, 2917−2921.

(16) Alaimo, L. H.; Ho, C.-T.; Rosen, J. D. Effect of protein glycation on subsequent volatile formation. *J. Agric. Food Chem.* **1992**, *40*, 280−283.

(17) Yaylayan, V. A.; Kaminsky, E. Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model system. *Food Chem.* **1998**, *63*, 25−31.
(18) Schroedter, R.; Baltes, W. Pyrolysis of various furan model compounds. *J. Anal. Appl. Pyrolysis* **1991**, *19*, 131−137.
(19) Takeoka, G.; Perrino, C.; Buttery, R. Volatile constituents of used frying oils. *J. Agric. Food Chem.* **1996**, *44*, 654−660.
(20) Tressl, R.; Kersten, E.; Nittka, C.; Rewicki, D. Mechanistic studies on the formation of pyrroles and pyridines from [1-^{13}C]-D-arabinose. In *Maillard Reactions in Chemistry, Foods and Health*; Labuza, T. P., Reineccius, G. A., Monnier, V. M., O'Brien, J., Baynes, J. W., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1994; pp 51−60.
(21) Basiuk, V. A.; Douda, J. Pyrolysis of poly-glycine and poly-L-alanine: analysis of less-volatile products by gas chromatography/Fourier transform infrared spectroscopy/mass spectrometry. *J. Anal. Appl. Pyrolysis* **2000**, *55*, 235−246.
(22) Wnorowski, A.; Yaylayan, V. A. Influence of pyrolytic and aquous-phase reactions on the mechanism of formation of Maillard products. *J. Agric. Food Chem.* **2000**, *48*, 3549−3554.
(23) Ferretti, A.; Flanagan, V. P. The lactose casein (Maillard) browning system: volatile compounds. *J. Agric. Food Chem.* **1971**, *19*, 245−249.
(24) Madruga, M. S.; Mottram, D. S. The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *J. Sci. Food Agric.* **1995**, *68*, 305−310.

Received for review December 10, 2001. Revised manuscript received March 13, 2002. Accepted March 14, 2002. We are indebted to the Fund for Scientific Research−Flanders (Belgium) (F.W.O.-Vlaanderen) and the I.W.T. for financial support.

JF0116247