

Volatile Compounds from the Reaction of Cysteine, Ribose, and Phospholipid in Low-Moisture Systems

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The headspace volatiles from Maillard reaction mixtures of cysteine and ribose heated at 185 °C with or without the addition of phosphatidylcholine, either dry or in the presence of a small quantity of water, were analyzed by GC-MS. The major products of the reaction were 3,5-dimethyl-1,2,4-trithiolane, 3-methyl-1,2,4-trithiane, 3,6-dimethyl-1,2,4,5-tetrathiane, and thieno[2,3-*b*]thiophene. Other products included a number of thiazoles and small amounts of some furan thiols and disulfides. Water had a significant effect on the relative amounts of volatiles produced; in particular, 1-(2-furanylmethyl)-1*H*-pyrrole, two other 1-(2-furanylmethyl)alkyl-1*H*-pyrroles, and two bis(furan) compounds were only produced to any extent in systems without water. The addition of lipid had only a small effect on the volatile profile, with small amounts of lipid degradation products and lipid/Maillard interaction products formed. These results differ greatly from previous studies on the volatiles formed in reaction systems carried out in the presence of large quantities of water.

Keywords: *Volatiles; Maillard reaction; moisture content; phospholipid*

INTRODUCTION

The two most important series of reactions for the formation of volatile aroma compounds in cooked and thermally processed foods are the Maillard reaction (between amino compounds and reducing sugars) and lipid degradation (thermal and oxidative). A number of early patents noted that lipid could influence the flavor of amino acid-sugar mixtures in reaction product flavorings (MacLeod and Seyyedain-Ardebili, 1981). However, the significance of Maillard/lipid interactions in the production of cooked food flavors was not fully recognized until the late 1980s. In a series of articles Whitfield, Mottram, and Farmer showed that a range of flavor compounds, particularly those with heterocyclic nuclei, were formed in interactions between amino acids, the pentose sugar, ribose, and phospholipids (Farmer and Mottram, 1990a; Farmer et al., 1989; Salter et al., 1988; Whitfield et al., 1988). All of these reactions were performed in aqueous solutions with the concentrations of the reactants similar to those found in raw meat. In a recent review of this work (Whitfield, 1992) it was suggested that future studies in this field should examine Maillard/lipid interaction in nonaqueous systems such as those encountered in roasting and extrusion.

The current study was undertaken to observe the effect that the absence of water had on the volatile products formed in the Maillard reaction between cysteine and ribose and on the Maillard/lipid interaction products.

EXPERIMENTAL PROCEDURES

Materials. L-Cysteine, D-(-)-ribose, and lecithin (L- α -phosphatidylcholine, type X-E from dried egg yolk) were

purchased from Sigma Chemical Co. Phosphate buffer (0.5 M, pH 5.6) was prepared from disodium hydrogen phosphate and sodium dihydrogen phosphate (BDH Chemicals Ltd.) in glass-distilled water. Authentic samples of volatile compounds were purchased from a range of laboratory chemical suppliers or were obtained as gifts from flavor laboratories.

Preparation of Reaction Mixtures. Mixtures of cysteine (10 mg) and ribose (10 mg) were prepared by grinding to fine powder in a mortar and pestle. The mixtures were transferred to small glass reaction tubes. Lecithin (40 mg) was added to one sample, water (100 μ L, buffered with phosphate at pH 5.6) was added to another, and both water and lecithin were added to a third, while a fourth sample contained only cysteine and ribose. The contents of the tubes were thoroughly mixed, and the tubes were flame sealed and then heated in an aluminum block at 185 °C for 20 min. Reactions of cysteine alone and cysteine with water were also carried out.

Isolation of Volatile Maillard Reaction Products. After cooling, each reaction mixture was transferred to a 250 mL conical flask containing 20 mL of 0.5 M phosphate buffer (pH 5.6) and a magnetic stirrer bar. The reaction tube was rinsed twice with buffer solution (2 mL), and these washings were added to the conical flask. The flask was fitted with a Dreschel head, and a glass-lined stainless steel tube (115 mm long \times 0.75 mm i.d.) packed with Tenax GC (Scientific Glass Engineering Pty Ltd., Australia) was attached by a stainless steel reducing union to the head outlet. During the collection of the volatile components, the dilute reaction solution was stirred slowly and maintained at 60 °C in a water bath, while the Tenax trap was maintained at room temperature. The volatiles were swept from the flask to the adsorbent in the trap using a flow of oxygen-free nitrogen (60 mL/min), and the collection was continued for 1 h. At the end of this time, the flask was removed and the trap was connected directly to the nitrogen supply for 5 min to remove moisture. An internal standard, 1,2-dichlorobenzene (65 ng, in 1 μ L of diethyl ether) was added to the front end of the trap just before GC-MS analysis.

Gas Chromatography-Mass Spectrometry. The gas chromatograph was a Hewlett-Packard HP5890 gas chromatograph, equipped with a "Unijector" (Scientific Glass Engineer-

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ing Pty Ltd., Australia) set in the concentrator-headspace mode and fitted with a fused silica capillary column (50 m long \times 0.32 mm i.d.) coated with DB-5 (Scientific Glass Engineering Pty Ltd., Australia). The gas chromatograph was directly coupled to a Hewlett-Packard HP5988A mass spectrometer. The helium carrier gas was set at a flow of 1 mL/min. The trapped volatile components were thermally desorbed onto the GC column by heating the trap at 250 °C for 5 min while the oven was cooled at 0 °C using the subambient facility. By directing the subambient cooling gas at the front of the column, efficient cryofocusing of the desorbed volatiles was achieved. After removal of the coolant, the column temperature was increased to 60 °C at a rate of 60 °C/min, held at that temperature for 5 min, and then increased to 250 °C at a rate of 4 °C/min. GC-MS of C_6 - C_{25} *n*-alkanes (100 ng of each in 1 μ L of diethyl ether added to the trap), analyzed under the above conditions, were used as external standard references for the calculation of linear retention indices of the volatile reaction products.

The mass spectrometer was operated in the electron impact mode with an electron energy of 70 eV and an ion source temperature of 250 °C. A continuous scan mode was employed with a scan time of 1 s over a mass range of 20–400 amu. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/MSDC and Wiley Mass Spectral Databases, in collections of mass spectra of flavor compounds (ten Noever de Brauw et al., 1980) or in previously published literature. Where possible, confirmation of identifications was carried by comparing of linear retention indices with those of authentic compounds.

RESULTS AND DISCUSSION

The volatiles from the reaction mixtures were dominated by sulfur-containing heterocyclics, with thiazoles, trithiolanes, and trithianes the most abundant. Table 1 lists 52 compounds identified from the cysteine/ribose reaction systems with or without phospholipid, carried out in wet or dry conditions. Thirty-nine of these compounds contained sulfur, and 10 compounds were only present in the lipid-containing systems and were derived from lipid degradation or lipid/Maillard interactions. To obtain some measure of the relative concentrations of these compounds in the different reaction mixtures, peak areas in each total ion chromatogram were compared with that of the dichlorobenzene internal standard (65 ng). Peaks that were $>150\%$ of the internal standard were defined as present in extra large quantities (++++), those between 50 and 150%, large quantities (+++), those between 10 and 50%, medium quantities (++) , those between 1 and 10%, small quantities (+), and those $<1\%$, trace quantities. A number of components remain unidentified, but none of these showed major differences between the reaction systems.

Cysteine was thermally stable when heated alone under dry conditions; however, heating cysteine on its own in the presence of a small amount of water led to the formation of a number of the above compounds, including most of the thiazoles, the thienothiophenes, and the polysulfur heterocyclics found in the cysteine/ribose systems. The quantities were significantly lower, as might be expected, since the breakdown of amino acids in the Maillard reaction occurs more readily than thermal degradation in the absence of a sugar. Recently, Zhang et al. (1988) observed the formation of trithiolanes, trithianes, and tetrathianes in the thermal degradation of cysteine. However, they reported very few thiazoles but significant quantities of thiadiazines and dithiazines. None of these latter compounds were found in the present reaction of cysteine alone; however, the pH was lower and the water content was much less

in the present work, and these variations in conditions may explain the differences between the two systems.

It is particularly interesting to compare the data for the present cysteine/ribose reactions with those obtained from earlier studies that used dilute aqueous solutions of cysteine and ribose heated at 140 °C (Farmer and Mottram, 1990a, 1992; Farmer et al., 1989; Whitfield et al., 1988). The present work was carried out either in the absence of water or with a relatively low moisture content (5:1, water:solids) and was performed at a higher temperature (185 °C). The conditions chosen were similar to those used for roasting coffee or those attained on the surface of grilled meat. There was a marked difference in the results obtained from these systems, i.e. containing no water or low water concentrations, compared with the aqueous systems investigated previously.

In reactions between cysteine, ribose, and lecithin under aqueous conditions a total of 152 compounds were identified, including many present in trace quantities. Only 34 of these were among the 52 compounds identified in the current study. Thus, 18 compounds, principally heterocyclic compounds containing sulfur, were not formed under aqueous conditions. Larger quantities of reactants were used in the aqueous studies, which may have accounted for the large numbers of trace components, but the absence of so many of the components that have now been found in the low-moisture systems illustrates the importance of reaction conditions on the formation of volatile products in the Maillard reaction.

In the previously reported aqueous systems, in the absence of lipid, the major volatile components were thiols such as 2-methyl-3-furanthiol, 2-furanmethanethiol, 2-thiophenethiol, 2-methyl-3-thiophenethiol, 2-mercapto-3-butanone, and two mercaptopentanones. Other major components were thienothiophenes, several thiophenones, and other oxygenated thiophenes. In the corresponding dry or low-moisture systems these compounds were not detected or were found at only trace levels, the only exception being the thienothiophenes, which were present in high concentrations. Other major components of the dry and low-moisture systems were 3,5-dimethyl-1,2,4-trithiolane, 3-methyl-1,2,4-trithiane, 3,6-dimethyl-1,2,4,5-tetrathiane, 1-(2-furanylmethyl)-1H-pyrrole, and 5-ethyl-4-methylthiazole. Although most of these were reported in the aqueous systems in significant amounts, they were not the dominant components. These current findings partly explain the differences in flavor observed between meats and other foods cooked under dry (e.g. grilled) or moist (e.g. boiled) conditions.

The addition of phospholipid to the dry and low-moisture system produced some equally unexpected results. Under the aqueous conditions of the previous work, large quantities of Maillard/lipid interaction products, including 2-pentylthiophene, 2-hexylthiophene, 2-pentylthiapyran, and 2-pentylpyridine, were produced together with large amounts of lipid oxidation products such as pentylfuran, alkanals, and alkanols. The low-moisture and dry systems reported in the present work contained only small amounts of these Maillard/lipid interaction products, and the only significant lipid oxidation products were a series of 2-alkanones. Clearly, when water is present in a very large excess, the volatile products of the Maillard reaction differ greatly from those of low-moisture systems. The conditions also affect the formation of lipid oxidation products, the dry

Table 1. Volatile Compounds Obtained from Reactions between Cysteine and Ribose in the Presence and Absence of Phospholipid and Water ^a

compound	LRI ^b	relative concentration ^c					method of identification ^d	MS data, <i>m/z</i> (rel intens), or literature reference ^e
		water absent		water present				
		C + R	C + R + L	C + R	C + R + L	C alone		
5-methylthiazole ^f	846	tr	—	tr	—	+	MS	ten Noever de Brauw et al. (1980)
2,4-dimethylthiazole ^f	892	tr	—	tr	—	—	MS + LRI	
4,5-dimethylthiazole	933	tr	—	tr	—	tr	MS + LRI	
5-ethylthiazole ^f	940	+	tr	—	—	tr	MS	ten Noever de Brauw et al. (1980)
2-propylthiazole ^f	981	tr	—	—	—	tr	MS	
trimethylthiazole	1000	+	+	+	+	+	MS + LRI	
5-ethyl-2-methylthiazole	1015	+++	++	+	++	+	MS	Farmer et al. (1989)
5-ethyl-4-methylthiazole	1039	tr	—	tr	tr	tr	MS + LRI	
2,5-dimethyl-4-ethylthiazole ^f	1066	+	tr	+	+	—	MS + LRI	
4-ethyl-5-methylthiazole	1068	tr	—	tr	—	—	MS	
2,4-dimethyl-5-ethylthiazole	1078	+	+	+	+	—	MS + LRI	
4,5-dimethyl-2-ethylthiazole ^f	1105	tr	tr	—	tr	—	MS + LRI	
4,5-dimethyl-2-propylthiazole ^f	1180	tr	+	tr	tr	tr	MS + LRI	
4(or 5)-ethyl-2-propylthiazole ^f	1190	+	+	tr	+	+	ms	127 (100), 140 (31), 71 (25), 112 (20), 85 (16), 45 (15), 126 (13), 39 (13), 128 (10), 155 (8), 154 (7)
1-(2-furanylmethyl)-1 <i>H</i> -pyrrole	1192	++	+++	—	tr	—	MS + LRI	
1-(2-furanylmethyl)methyl-1 <i>H</i> -pyrrole ^f	1276	tr	+	—	—	—	ms	81 (100), 53 (14), 161 (8), 82 (6), 27 (3), 97 (2), 117 (2)
1-(2-furanylmethyl)dimethyl-1 <i>H</i> -pyrrole ^f	1361	—	tr	—	—	—	ms	81 (100), 175 (22), 97 (7), 82 (6)
2,2'-methylenebis(furan) ^f	1086	tr	+	—	—	—	MS	
2,2'-(1,2-ethylenediyl)bis(furan) ^f	1336	++	++	—	tr	—	MS	
4,5-dihydro-2-methyl-3(2 <i>H</i>)-thiophenone	989	—	—	++	tr	—	MS + LRI	
2-acetylthiophene	1086	tr	—	tr	tr	tr	MS + LRI	
1-(2-thienyl)-1-propanone	1188	tr	—	tr	tr	—	MS + LRI	
thieno[2,3- <i>b</i>]thiophene	1212	++	++	+++	++++	++	MS	Farmer et al. (1989)
thieno[3,2- <i>b</i>]thiophene	1214	+	tr	+	+	tr	MS	ten Noever de Brauw et al. (1980)
3,5-dimethyl-1,2,4-trithiolane (<i>E</i> or <i>Z</i>)	1136	++	++	+++	++++	+	MS + LRI	
3,5-dimethyl-1,2,4-trithiolane (<i>E</i> or <i>Z</i>)	1148	++	++	+++	++++	+	MS + LRI	
3-methyl-1,2,4-trithiane	1243	++	++	++	++	+	MS + LRI	152 (100), 60 (94), 59 (87), 92 (53), 64 (44), 45 (36), 58 (13), 154 (12), 61 (11)
3,6-dimethyl-1,2,4,5-tetrathiane (<i>E</i> or <i>Z</i>) ^f	1388	++	++	+++	+++	++	MS	Zhang et al. (1988)
3,6-dimethyl-1,2,4,5-tetrathiane (<i>E</i> or <i>Z</i>) ^f	1406	+	+	++	++	+	MS	Zhang et al. (1988)
4,6-dimethyl-1,2,3,5-tetrathiane ^f	1442	+	—	+	+	+	MS	Zhang et al. (1988)
2-methyl-3-furanthiol	870	tr	—	tr	tr	—	MS + LRI	Evers et al. (1976)
2-furanmethanethiol	913	+	+	tr	—	—	MS + LRI	
2-methyl-3-thiophenethiol	1058	—	—	tr	—	—	MS	van den Ouweland and Peer (1975)
2-methyl-3-(methylthio)furan	947	—	+	—	—	—	MS	MacLeod and Ames (1986)
3-(methylthio)thiophene ^f	1082	—	++	—	tr	—	MS	ten Noever de Brauw et al. (1980)
2-methyl-3-(methylthio)thiophene ^f	1144	—	+	—	—	—	ms	129 (100), 144 (91), 45 (16), 85 (15), 97 (14), 131 (9), 69 (9), 146 (8)
bis(2-methyl-3-furanyl) disulfide	1547	+	—	++	tr	—	MS + LRI	Mottram et al. (1994)
3-(2-methyl-3-furanyldithio)pentan-2-one	1580	—	—	tr	—	—	MS + LRI	Mottram et al. (1994)
2-(2-methyl-3-furanyldithio)pentan-3-one	1593	—	—	+	—	—	MS + LRI	Mottram et al. (1994)
2-methyl-3-(2-furanylmethylthio)furan	1649	—	—	+	—	—	MS + LRI	Mottram et al. (1994)
bis(2-furanylmethyl) disulfide	1701	—	—	tr	—	—	MS + LRI	Mottram et al. (1994)
2-methyl-3-(2-methyl-3-furanyldithio)thiophene	1727	tr	—	+	tr	—	MS	Werkhoff et al. (1990)
2-heptanone	890	—	+	—	tr	—	MS + LRI	
2-octanone	993	—	+	—	tr	—	MS + LRI	
2-nonanone	1095	—	++	—	tr	—	MS + LRI	
2-decanone	1198	—	++	—	—	—	MS + LRI	
2-undecanone ^f	1297	—	+	—	—	—	MS + LRI	
1-octen-3-ol	981	—	++	—	tr	—	MS + LRI	
2-pentylfuran	991	—	+	—	—	—	MS + LRI	
2-pentylthiophene	1164	—	+	—	—	—	MS + LRI	
2-pentylthiapyran	1317	—	++	—	—	—	ms	97 (100), 98 (6), 168 (4), 45 (4), 111 (2)
2-pentylpyridine	1202	—	tr	—	—	—	MS + LRI	

^a C, cysteine; R, ribose; L, phospholipid (lecithin). ^b Linear retention index. ^c Relative size of peaks in GC-MS chromatogram: +++++, very large; +++, large; ++, medium; +, small; tr, trace; —, not detected. ^d MS + LRI, mass spectrum and LRI agree with those of authentic compound; MS, mass spectrum agrees with literature spectrum; ms, interpretation of mass spectrum. ^e Where neither mass spectrum nor a reference is given, the reference spectrum can be found in NIST/EPA/MSDC or Wiley Mass Spectral Databases. ^f Compound not found in previous work involving reaction in dilute aqueous solution.

and low-moisture systems producing many fewer lipid-derived volatiles.

A number of studies have investigated the role of moisture in the oxidation of lipids in food systems and, in general, oxidation decreases with decreasing water activity, although at activities below a_w 0.2–0.3 oxidation is higher than at the intermediate levels (Nelson and Labuza, 1992). The food matrix plays a significant role in determining these effects, and the ability of water to mobilize reactants and catalysts may be important. Other effects of water, such as hydrogen bonding to lipid hydroperoxides and the reaction with free radical oxidation intermediates, are also believed to be important. In the systems reported in this paper the mobility of reactants, especially the polar phospholipid, and intermediates in the aqueous systems may play an important role in determining the amount and type of lipid oxidation products.

A comparison within the systems used in the present study further demonstrates the importance of water in determining the nature of the volatiles produced in the Maillard reaction. The dry cysteine/ribose systems showed more thiazoles than the other systems and quantities were higher. In the absence of water, 1-(2-furanylmethyl)-1*H*-pyrrole was a major volatile component, the concentration of which was increased by the presence of lipid. Small amounts of other 1-(furanylmethyl)alkyl-1*H*-pyrroles were also found. However, with the addition of a small amount of water these compounds were almost eliminated from the volatiles. Similarly, the two bisfuran compounds were associated with the dry Maillard systems. Thiophenes and thienothiophenes were more readily formed in the presence of water, as were the trithiolanes, trithianes, and tetrathianes.

Among the most important contributors to the characteristic aroma of cooked meat are 2-methyl-3-furanthiol, the corresponding thiophene, and disulfides derived from these thiols (Mottram, 1991). These compounds have extremely low odor threshold values, and consequently only trace amounts are needed for meatlike aromas; indeed, at higher concentrations many of the compounds become unpleasantly sulfurous in character. Such thiols were found in trace quantities in the four reaction systems, and disulfides were only found to any extent in the water-containing system without phospholipid. Interestingly, the three methyl sulfides, 2-methyl-3-(methylthio)furan, 2-methyl-3-(methylthio)thiophene, and 3-(methylthio)thiophene, were only formed in systems containing phospholipid. Other reports have discussed the importance of pH and lipid in the formation of thiols and disulfides in meatlike model systems (Farmer and Mottram, 1990b; Mottram and Madruga, 1994; Mottram and Whitfield, 1994). The present work demonstrates that other conditions can influence their formation.

There are many different routes to the formation of aroma compounds in the Maillard and associated reactions, few of which have been fully elucidated. The results reported here clearly indicate that certain reactions are more favored in dry conditions, whereas others occur more readily in the presence of large amounts of water. For example, furan- and thiophenethiols, mercaptoketones, and other oxygenated sulfur heterocyclics, such as dithiolanones, are formed from the action of hydrogen sulfide on ribose-derived Maillard reaction products (Zhang and Ho, 1991; Mottram and Whitfield, 1994). On the other hand, 3,5-dimethyl-1,2,4-trithiol-

ane, 3-methyl-1,2,4-trithiane, and 3,6-dimethyl-1,2,4,5-tetrathiane can be formed from the interaction of acetaldehyde, hydrogen sulfide, and mercaptoacetaldehyde, all of which are produced by the breakdown of cysteine at high temperature. At low moisture levels the thermal degradation of cysteine is much more prominent than reactions of ribose with hydrogen sulfide, which requires relatively high water content.

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