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Article

The Effect of pH on the Formation of Volatile Compounds Produced by Heating a Model System Containing 5’-Imp and Cysteine

***Marta Suely Madruga*[a], *and Donald S. Mottram*[b]**

[a]*Departamento de Tecnologia Química e de Alimentos (DTQA),*
Universidade Federal da Paraiba, UFPB, Campus I, Cidade Universitária,
58059-900 João Pessoa - PB, Brazil
[b]*Department of Food Science and Technology, University of Reading, Whiteknights,*
Reading RG6 6AP, England, UK

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A identificação de compostos voláteis formados a partir de reações entre Inosina-5’-Monofosfato (5’-IMP) e cisteína a três diferentes pH (3,0; 4,5; 6,0) e 140 °C foi realizada através da análise de “headspace” dinâmico. Os resultados mostraram que mais de 90 compostos voláteis foram produzidos, principalmente compostos heterocíclicos, incluindo furanos sulfurados, tiofenos, tiazoles, furanos, sulfitos alquilados, compostos bicíclicos e sulfitos cíclicos. Os estudos demonstraram que os furanos sulfurados, mercaptocetonas e alquil-furanos foram formados principalmente a pH ácido, enquanto que as pirazinas foram completamente inibidas a pH elevado. Estes resultados confirmam observações preliminares de que o pH exerce uma grande influência nos compostos voláteis formados em reações de Maillard.

The identification of volatile compounds formed from the reactions of Inosine-5’-Monophosphate (5’-IMP) with Cysteine at three different pH (3.0; 4.5; 6.0) and 140 °C were performed using dynamic headspace analysis. The results gave over 90 volatile compounds, mainly heterocyclic compounds, including sulphur containing furans, thiophenes, thiazoles, furans, alkyl sulphides, bicyclic compounds and cyclic sulphides. The studies showed that sulphur-substituted furans, mercaptoketones and alkylfurans were formed mainly at acidic pH, while pyrazines were completely inhibited at high pH. These findings support an earleir observation that pH has a great influence on volatile compounds formed in Maillard type reactions.

Keywords: *volatile compounds, pH, cysteine, inosine-5’-monophosphate*

Introduction

It has been established that the Maillard reaction between pentose sugars and cysteine or hydrogen sulphide is very important for meat flavour[1,2,3]. The main source of pentoses in meat are ribonucleotides and 5’-IMP is the principal ribonucleotide in post-mortem meat. The reaction between pentose and cysteine has been examined in model systems[4,5,6]. It was also observed that pH influenced such reactions[7,8] and that meat flavour formation also seems to be pH dependent[9]. Work has been done on the thermal stability of nucleotides in aqueous solutions and the thermal reaction of 5’-IMP with amino acids at pH 2.3 has been studied[10]. However the influence of different pHs on the volatile products from reactions of nucleotides with amino acids has not been studied. To better elucidate the participation of 5’-IMP and cysteine, as well as pH, in the formation of some important meat flavour compounds, heated model systems were studied using the reactants, 5’-IMP and cysteine, at three different pHs, *i.e.*, 6.0, 4.5 and 3.0.

Material and Methods

Preparation of reaction mixtures

Reactions between 5'-IMP and cysteine were carried out at three different pHs, *i.e.*, 3.0, 4.5 and 6.0. Cysteine (0.121 g) and 5'-IMP (0.348 g) were dissolved in 10 mL of an appropriate pyrophosphate buffer (pH 3.0; 4.5, 6.0). One millilitre (1.0 mL) aliquots of each solution were transferred to the pyrex ampoules, nitrogen was blown over each sample for 3 min, and the ampoules flame-sealed. The final concentrations in each reaction were 12.1 mg/mL and 34.8 mg/mL of cysteine and 5'-IMP respectively. These concentrations were related to the amount of these compounds in meat muscle[11]. The reactions were carried out in triplicate. Ampoules containing the reaction mixtures were placed in a horizontal position in a CERTOclav autoclave (Kelomat, Traun, Austria) and heated for 1 h at 140 °C under a pressure of 0.28 MPa (2.7 bar). After the reaction, the mixtures were left to cool and then submitted to headspace analysis. A pH measurement was carried out before and after each heat treatment. In general the pH of reaction mixtures decreased by less than 0.2 pH unit after heating.

Methods

Headspace Collection of volatiles

For the collection of the volatile compounds produced by heat treatment, a dynamic headspace technique was used. It was similar to that described by Madruga and Mottram[12],and Madruga[13]. The cooled ampoules were broken manually and the samples were immediately transferred to a 250 mL conical flask fitted with 30mm screw joints to take a sliding joint with PTFE seal (SVL fittings; J. Bibby Science Products, Stone) and Dreschel head. Pyrophosphate buffer (20 mL) at the appropriate pHs were added to the reaction mixtures and aimed to dilute the mixtures. The volatiles were collected in a glass-lined stainless steel trap (155 mm long x 0.75 mm id) packed with Tenax-GC (SGE Ltd). During the collection of the volatile components, the conical flask was maintained at 60 °C, in a water-bath with constant agitation. The volatiles were swept on to the adsorbent in the trap using a flow of oxygen-free nitrogen (40 mL/min) and collected during 1 h. The oxygen-free nitrogen supply was purified by passing through granular charcoal to remove traces of organic compounds. The flow was adjusted via a pressure regulator and a mass flow controller. At the end of the volatile collection, the flask was removed and the trap was connected directly to the nitrogen supply for 5min, to remove moisture. Usually no less than three headspace collections were performed for each experiment.

Gas chromatography with FID detection and odour assessment

After collection, the volatiles were thermally desorbed, using a modified injector port, directly on to the front of a DB-5 (30 m x 0.32 mm id x 1.0 µm film, J & W Scientific Inc) or a CP-Wax-52CB 52CB (50 m x 0.32 mm id x 0.21 µm film, Chromapak UK Ltd) fused silica capillary column, in the oven of a Hewlett-Packard HP5890 Gas Chromatograph. The oven was held initially at 0 °C for 5 min while the the volatiles were desorbed from the Tenax trap (held at 250 °C in the modified injector). After the removal of the coolant, the column temperature was rapidly increased to 60 °C and maintained at this temperature for 5 min. Then it was ramped at 4 °C/min to the final temperature, where it was held for a further 20 min. For the DB-5 column the final temperature was 250 °C, but a lower temperature of 220 °C was used with the less-stable CP-Wax column. At the end of the column, the effluent was split 1:1, into two deactivated fused silica capillaries (0.4 mm od; 0.2 mm id) of equal length, using a 1/16 in. stainless steel union with a two-hole ferrule carrying the two fused silica capillaries (SGE Ltd).

One of the ends was connected to the FID, and the other was pushed through a length of glass-lined stainless steel tubing (1/16 in id; 0.7 mm id) (SGE Ltd). This tubing passed through the GC detector heater block and into a glass nose connection, which was used as the "sniffing port" mounted above in the GC. Another line was connected to the sniffing port, carrying air, which was moistened by bubbling it through distilled water. For each analysis the aroma of the effluents was assessed by four individuals, with experience in flavours, who marked the chromatogram and noted a description for each aroma detected. A clean nose-cone was fitted for each assessor. Helium at 2 mL/min was used as carrier gas.

Determination of linear retention indices (LRI)

A standard mixture of n-alkane (C_6 - C_{20}) in ethanol was analyzed each day before GC runs to allow a check of the instrument performance and the calculation of retention indices of each component in the samples. The standard (1.0 µl) was injected to the trap and the solvent was removed by purging with oxygen-free nitrogen (40 mL min^{-1}) for 5 min. These alkanes were used as external standard references in Linear Retention Indice (LRI) calculations. LRI of each compound was calculated from the standard alkane retention time and the peak retention time using the Eq. 1.

$$\mathrm{LRI} = \frac{[RT_x - RT_n + n]}{RT_{n+1} - RT_n} \times 100 \qquad (1)$$

where:

LRI = Linear Retention Index

RT_x = retention time of compound
RT_n = retention time of n-alkane before peak
RT_{n+1} = retention time of n-alkane after peak
n = carbon number of n-alkane before peak

Gas chromatography-mass spectrometry (GC-MS)

Analyses were performed on a Hewlett Packard 5988A Mass Spectrometer with a HP5890 Gas Chromatograph, linked to a HP 59970 GC/MS Workstation. The GC columns and conditions used were the same as those described above. The following conditions were used for the mass spectrometer; source temperature = 200 °C, ionising voltage = 70 eV); scan range from m/z 29-290, with 1.44 scans per second

Results and Discussion

The major headspace components of the model system involving 5'-IMP + cysteine at three different pHs (3.0, 4.5, 6.0) are listed in Table 1 in order of elution. The 98 compounds presented are those which gave significant peaks in gas chromatograms, together with minor components of possible odour significance, *e.g.* compounds which were described as having a meat-like aroma or some similar description. The identity of 56 of these have been established by comparison of mass spectral data and Linear Retention Indices (LRI) with those of authentic materials. Thirty-nine identities have been suggested for compounds whose mass spectra agree with literature spectra, but without reference to LRI, and three other compounds (sulphur containing structures) were suggested by the interpretation of mass spectra and comparison with those of related compounds. Eight compounds (in italics) have been synthesised[13]. The gas Chromatogram of headspace volatiles collected from 5'-IMP/cysteine model system heated at pH 3.0, in a DB-5 column is presented in Fig. 1.

The volatiles identified in these three reaction mixtures were dominated by sulphur-containing compounds, especially disulphides and thiophenes. Also thiazoles, alkyl sulphides, cyclic sulphides, bicyclic compounds, furans and ketones were found. The total quantity of volatile compounds increased significantly as the pH decreased. On both columns, a large number of heterocyclic disulphides were identified from the reaction of 5'-IMP with cysteine at pH 4.5 and 3.0, but at pH 6.0 only one was detected.

Odour port evaluation of the volatiles, as they eluted from the GC column, showed eighteen components which appeared to have meaty and similar aromas. Many of these were identified as disulphides and some have been previously identified as important contributors to the odour of meat[12,14,15,16,17]. Furans and thiophenes with a thiol group in the 3-position appear to have meat-like aromas, as do the disulphides formed by their oxidation[14,18,19]. In general, compounds containing the 2-methyl-3-furyl group possessed meaty aromas, while compounds with the 2-furylmethyl and 2-thienyl groups were more roast and rubbery.

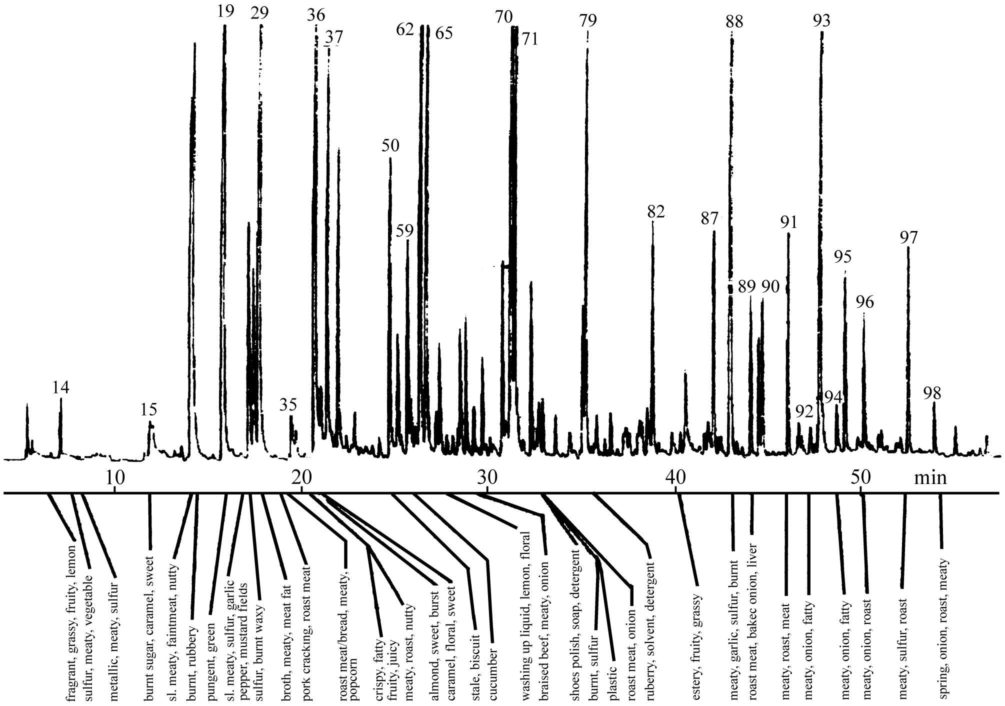

Figure 1. Gas Chromatogram of headspace volatiles collected from 5'-IMP/cysteine model system heated at pH 3.0, showing a summary of the aromas detected in the polar column effluent. Peak numbers related to compounds in Table 1.

Table 1. Compounds identified in the volatiles from the reaction between 5'-IMP and Cysteine, at different pHs, using DB-5 and CP-Wax columns.

No	Compound[a]	pH[b]			LRI[c]		Method of identification[d]	MS data
		6.0	4.5	3.0	DB-5	CP-WAX		
1	sulphur dioxide	+	+	+	-	-	MS[1]	**64**, 48(40), 65(5), 66(10)
2	carbon disulphide	+	+	+	-	-	MS[1]	**76**, 44(12), 78(10), 64(5)
3	2-butanone	+	+	nd	603		MS[2]	43, **72**(18), 42(8), 44(5), 45(5), 39(4), 57(4)
4	ethyl acetate	+	+	nd	612		MS[1]	43, 45(14), 61(12), 70(11), 42(9), 73(5),**88**(4)
5	benzene	+	nd	nd	664	930	MS+LRI	
6	2-pentanone	+	+	+	689	974	MS+LRI	
7	3-pentanone	+	+	nd	694		MS[1]	57, **86**(17), 43(10), 58(10), 56(4)
8	2,3-pentanedione	+	nd	+	725		MS+LRI	
9	3-penten-2-one	nd	nd	+	728		MS[1]	41, 69(90), 43(70), **84**(31), 42(12)
10	2-methylthiophene	+	+	+	777	1092	MS+LRI	
11	2,4-pentanedione	nd	+	+	782		MS+LRI	
12	2-hexanone	+	+	nd	798	1095	MS+LRI	
13	hexanal	+	+	+	799	1097	MS+LRI	
14	*3-mercapto-2-butanone*	+	+	+	817		MS+LRI	
15	2-furfural	+	+	+	829	1457	MS+LRI	
16	2-methylthiazole	+	+	+	832	1250	MS+LRI	
17	2-methylcyclopentanone	+	nd	nd	836	1366	MS+LRI	
18	2,4-dimethylfuran	+	nd	nd		1535	MS[2]	**96**, 67(97), 53(33), 39(20), 81(7)
19	2-methyl-3-furanthiol[e]	nd	+	+	870	1294	MS+LRI	
20	2-ethylthiophene	+	+	+	871	1170	MS+LRI	
21	4-hydroxy-5-methyl-3(2H)-furanone	nd	+	nd	880		MS+LRI	
22	2,5-dimethylthiophene	+	+	+	882		MS+LRI	
23	2-heptanone	+	+	+	890	1180	MS+LRI	
24	2-butylfuran	+	+	+	893		MS+LRI	
25	3-heptanone	+	nd	nd	895		MS[1]	57, 85(41), 41(30), 72(20), 43(12), **114**(10)
26	3-mercapto-2-pentanone	nd	+	+	898	1347	MS+LRI	
27	2-acetylfuran	nd	+	+	904	1498	MS+LRI	
28	*2-mercapto-3-pentanone*	nd	+	+	910		MS+LRI	
29	2-furylmethanethiol[e]	nd	+	+	913	1420	MS+LRI	

30	3 or 4-methyl-2-furfural	nd	nd	+	920		MS[5]	**110**, 109(90), 53(69), 51(23), 81(19), 51(10), 43(10), 39(8), 111(7)
31	dimethylsulphone	+	+	+	925		MS[1]	79, **94**(50), 45(12), 48(6), 63(6), 81(5)
32	5-methyl-2-furfural	nd	+	+	949	1572	MS+LRI	
33	4,5-dihydro-3(2H)-thiophenone	nd	+	+	952		MS+LRI	
34	1-(2-furyl)-2-propanone	nd	nd	+	954	1512	MS[2]	81(100), 43(56), 53(33), **124**(33), 82(30), 51(7), 52(6), 50(5), 54(4), 39(4)
35	benzaldehyde	+	+	+	958	1518	MS+LRI	
36	2-methyl-4,5-dihydro-3-furanthiol[e]	nd	nd	+	975		MS[3]	**116**, 43(74), 73(48), 45(40), 101(20), 71(25), 72(20), 74(16), 39(14), 69(11), 58(5)
37	2- or 3-thiophenethiol[e]	+	+	+	977	1554	MS+LRI	
38	4,5-dihydro-5-methyl-3(2H)-thiophenone	nd	+	+	978		MS+LRI	
39	5-methyl-2-acetylfuran	nd	nd	+	985	1622	MS+LRI	
40	4,5-dihydro-2-methyl-3(2H)-thiophenone	+	+	+	990	1520	MS+LRI	
41	2-pentylfuran	nd	+	nd	994		MS+LRI	
42	2,4,5-trimethylthiazole	+	+	+	996	1390	MS+LRI	
43	2-formylthiophene	+	+	+	1004	1687	MS+LRI	
44	4-ethyl-5-methylthiazole	+	+	+	1005	1412	MS+LRI	
45	2-propionylfuran	nd	nd	+	1019		MS+LRI	
46	2-ethyl-1-hexanol	+	+	+	1025	1490	MS+LRI	
47	1,3-dithiane	nd	nd	*	1027		MS[5]	**120**, 45(15), 72(10)
48	a dimethylfurfural	nd	+	+	1040	1610	MS[7]	123, **124**(88), 67(21), 39(15), 41(13), 95(9), 65(9), 125(7), 91(6), 43(6), 53(5), 51(4)
49	5-methyl-3(2H)-thiophenone	nd	*	*	1042		MS[1]	**114**(90) ,86(64), 71(53), 59(43), 53(29), 45(27), 85(22), 55(19), 39(18), 116(13), 115(13), 69(13), 58(12), 50(12), 51(11), 99(9)
50	2-methyl-3-thiophenethiol[e]	nd	+	+	1065	1580	MS+LRI	
51	1-(2-furyl)-3-butanone	nd	nd	+	1070	1640	MS[2]	81, 109(87), 43(77), **138**(68), 95(65), 53(20), 67(18), 68(12), 123(11), 39(9), 94(8), 96(8), 110(7), 66(7)
52	3-methyl-1,2-dithiolan-4-one	nd	+	+	1072	1664	MS+LRI	
53	2,5-dimethyl-4-ethylthiazole	nd	nd	+	1074		MS+LRI	
54	2-thiophenemethanethiol[e]	nd	nd	+	1089	1689	MS+LRI	
55	2-acetylthiophene	nd	nd	+	1090	1773	MS+LRI	

56	a formylmethylthiophene	+	+	+	1093	1712	MS^5	**125**, 126(96), 97(58), 53(30), 45(27), 39(13)
57	(EorZ) 3,5-dimethyl-1,2-dithiolan-4-one	nd	+	+	1098	1568	MS^5	60, **148**(65), 59(48), 92(36), 55(20), 64(15), 45(10), 61(9), 56(9), 120(6)
58	(EorZ) 3,5-dimethyl-1,2-dithiolan-4-one	nd	+	+	1111	1597	MS^5	60, **148**(65), 59(48), 92(36), 55(20), 64(15), 45(10), 61(9), 56(9), 120(6)
59	2-formyl-5-methylthiophene	+	+	+	1118	1783	MS+LRI	
60	2-formyl-3-methylthiophene	+	+	+	1121	1815	MS+LRI	
61	1,2,4-trithiolane	*	*	*	1127		MS^2	45, 78(96), **124**(73), 46(50), 60(33), 80(25), 44(21), 57(11)
62	(E) 3,5-dimethyl-1,2,4-trithiolane	+	+	+	1140	1580	MS+LRI	
63	2-acetyl-5-methylthiophene	nd	nd	+	1145	1874	MS+LRI	
64	an ethylmethylfurfural	nd	+	+	1147	1675	MS^5	**138**, 123(75), 137(23), 109(22), 95(22), 41(20), 81(20)
65	(Z) 3,5-dimethyl-1,2,4-trithiolane	+	+	+	1151	1603	MS+LRI	
66	2-acetyl-3-methylthiophene	nd	nd	+	1161	1762	MS+LRI	
67	3-ethyl-1,2-dithiolan-4-one	nd	+	+	1163		ms	**148**, 74(63), 106(43), 41(40), 45(30), 64(22)
68	2-propionylthiophene	nd	nd	+	1181	1840	MS+LRI	
69	naphthalene	+	+	+	1185	1730	MS+LRI	
70	3-ethyl-2-formylthiophene	+	+	+	1203		MS+LRI	
71	3,(5or6)-dimethyl-1,2-dithian-4-one	nd	nd	+		1853	MS^5	60, **162**(70), 92(64), 55(48), 120(39), 64(30), 41(29), 42(27), 56(26), 74(15), 59(12)
72	2,3-dihydro-6-methylthieno[2,3c]furan	+	+	+	1209		MS+LRI	
73	3-methyl-1,2-dithian-4-one	nd	+	+	1213	1896	MS^4	**148**, 60(94), 64(35), 92(30), 55(22), 89(17), 45(17), 59(10)
74	2-(2-thienyl)-furan	nd	nd	+	1227		MS^8	**150,** 121(70), 122(36), 96(21), 78(20), 45(20), 77(19)
75	benzothiazole	*	nd	*	1231		MS^2	**135**, 108(36), 69(25), 82(10), 63(10), 91(9)
76	a dimethylformylthiophene	+	+	+	1240	1915	MS^5	139, **140**(75), 111(19), 67(16), 45(10), 39(9)
77	3-methyl-1,2,4-trithiane	+	nd	nd	1253	1862	MS+LRI	
78	a thienothiophene	+	+	+	1257	1860	MS^2	**140**, 96(23), 58(21), 45(18), 69(16), 141(12), 142(11), 82(9), 51(8)
79	a dihydrothienothiophene	+	+	+	1284	1958	MS^5	**142**, 141(88), 97(60), 116(45), 71(33), 45(25), 69(25), 58(12), 143(11), 144(10)
80	a methylthienothiophene	nd	+	+	1322	1943	MS^5	153, **154**(77), 155(13), 69(12), 77(11), 45(8)
81	1,2,4,5-tetrathiane	+	+	nd	1337		MS^2	64, **156**(88), 128(67), 45(38), 59(35), 60(34), 92(32), 124(28), 96(19)

82	a methyldihydrothienothiophene	nd	+	+	1341	2003	MS[5]	**156**, 155(69), 111(28), 141(25), 59(20), 45(17), 97(15), 157(15), 158(12)
83	a methylthienothiophene	nd	+	+	1364	1957	MS[5]	153, **154**(77), 155(13), 69(12), 77(11), 45(10), 39(9), 156(8)
84	a methyldihydrothienothiophene	nd	+	+	1417	2017	MS[5]	**156**, 155(67), 111(32), 141(24), 59(16), 97(11), 153(11), 158(10), 154(10), 140(10), 122(8), 123(8), 45(8), 77(7), 69(7)
85	2-ethylthienothiophene	nd	+	+	1422	2012	MS[2]	153, **168**(43), 69(20), 45(15), 141(13), 121(8)
86	3,4,6-trimethyl-1,2,5-trithiane	+	+	+	1499		MS[2]	59, 60(73), 45(25), 55(20), 61(19), **180**(15), 58(12), 129(10)
87	*2-methyl-3-furyl 1-methyl-2-oxopropyl disulphide*[e]	nd	nd	+	1505	2130	MS+LRI	113, 43(79), **216**(36) ,114(29), 59(18), 45(14), 81(13), 173(11), 217(6), 218(5)
88	*bis(2-methyl-3-furyl) disulphide*[e]	nd	+	+	1540	2110	MS+LRI	113, **226**(44), 43(30), 114(14), 69(13), 85(12)
89	*2-methyl-3-furyl 1-ethyl-2-oxopropyl disulphide*[e]	nd	nd	+	1574	2233	MS+LRI	113, 43(92), **230**(43), 114(34), 145(27), 85(15), 81(14), 187(13), 231(6), 232(5)
90	*2-methyl-3-furyl 1-methyl-2-oxobutyl disulphide*[e]	nd	+	+	1596	2265	MS+LRI	113, 57(78), **230**(37), 114(32), 43(29), 173(14), 85(12), 145(7), 23(5), 232(4)
91	*2-methyl-3-furyl 2-furylmethyl disulphide*[e]	nd	+	+	1640	2347	MS+LRI	81, 113(18), 53(18), 43(14), **226**(9), 45(12), 85(11), 162(1)
92	*bis(2-furylmethyl) disulphide*[e]	nd	nd	+	1683		MS+LRI	81, 53(14), **226**(7), 69(5), 112(4), 193(2)
93	2-methyl-3-furyl 2- or 3-thienyl disulphide[e]	nd	+	+	1699	2390	MS[6]	43, **228**(56), 45(37), 115(26), 81(23), 85(25), 164(11), 185(10)
94	2- or 3-thienyl 1-methyl-2-oxobutyl disulphide[e]	nd	nd	+	1732		ms	43, 71(45), 116(45), 147(40), **232**(30), 45(37), 115(25), 39(16), 41(13), 189(12), 105(12), 69(12), 103(11), 84(10), 58(8)
95	2-methyl-3-furyl 2-methyl-3-thienyl disulphide[e]	nd	+	+	1751	2395	MS[6]	129, 113(96), 45(82), **242**(70), 85(47), 97(27), 199(15)
96	2-furylmethyl 2- or 3-thienyl disulphide[e]	nd	+	+	1789		MS[6]	81, 45(14), 53(14), 71(8), 115(5), 116(4), 85(4), **228**(3)
97	bis(2- or 3-thienyl) disulphide[e]	nd	+	+	1871		ms	115, 71(70), 45(46), **230**(41), 116(26), 166(22), 69(17), 89(10), 39(10), 232(8), 197(6)
98	2-methyl-3-thienyl 2- or 3-thienyl disulphide[e]	nd	+	+	1923	2715	MS[6]	129, 45(63), 71(44), **244**(37), 116(23), 69(23), 115(22), 85(20), 97(19), 59(15), 131(10)

a compounds in italics have been syntesised.

b + compound gives clear MS; nd not detected; * compound gives no clear MS, detected mixed with other volatile

c LRI - Linear Retention Index.

d Methods of identification: MS+LRI - mass spectrum and LRI agree with those of authentic compounds analysed under similar conditions
MS - mass spectrum agrees with literature spectra: 1. NIST/EPA/MSDC[44]; 2. ten Nover de Brauw *et al.*[45]; 3. van den Ouweland & Peer[25]; 4. Hartman *et al.*[39]; 5. Farmer *et al.*[4]; 6. Farmer & Mottram[7]; 7. Salter *et al.*[46]; 8. Werkhoff *et al.*[30]; ms - interpretation of mass spectrum and comparison with those of related compounds

e compounds appeared to have meaty and similar aromas.

These data show some agreement with those from related systems reported in the literature. Zhang & Ho[10] identified 41 sulphur containing compounds in a total of 47 compounds from a model system containing 5'-IMP and cysteine at pH 2.3. Farmer *et al.*[4] reported formation of 115 volatiles, mainly heterocyclic compounds, from the reaction between cysteine and ribose at pH 5.7 using conditions similar to ours. Mulders[5] reacted cysteine (and cystine) with ribose at pH 5.6 and reported 40 heterocyclic compounds. Forty-seven compounds were reported from the reaction between cystine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone heated at pH 2.4[20].

The sulphur-containing furans/ thiophenes and related disulphides were among the most numerous classes of flavour compounds formed in these reaction mixtures, although many were present at low concentrations. Eighteen and twelve compounds were identified in 5'-IMP/cysteine mixtures at pH 3.0 and 4.5 respectively, however only one, 2- or 3-thiophenethiol (compound n [**37**] in Table 1), was also detected in the reaction mixture at pH 6.0. These results confirm previous observations that the formation of furyl and thienyl sulphides and disulphides is extremely pH dependent, with maximum formation at low pH values[7,8,12,16,17,21,22,23,24]. The thiols identified included three furanthiols (2-methyl-3-furanthiol [**19**], 2-methyl-4,5-dihydro-3-furanthiol [**36**], 2-furylmethanethiol [**29**]) and three thiophenethiols (2- or 3-thiophenethiol [**37**], 2-methyl-3-thiophenethiol [**50**], 2-thiophenemethanethiol [**54**]).

The compounds **19** and **36** were probably formed in the reaction of hydrogen sulphide (Strecker degradation product of cysteine) with 4-hydroxy-5-methyl-3(2H)-furanone (a dehydration product of ribose or 5'-IMP) or other pentose dehydration products[13,25]. 2-Furylmethanethiol [**29**] was probably formed by the reaction of hydrogen sulphide with 2-furfural, another ribose and 5'-IMP breakdown product[26]. 2-Methyl-3-thiophenethiol [**50**], identified in these systems, was probably formed from α-dicarbonyls or furanones by reaction with H_2S[25,27]. Farmer & Mottram[7] found that the levels of 2-methyl-3-furanthiol [**19**] and 2-methyl-3-thiophenethiol [**50**] decreased with increasing pH in a reaction mixture of cysteine and ribose. 2-Thiophenemethanethiol [**54**] has been reported as a product of the reaction of H_2S and 2-furfural[26]. The 2- or 3-thiophenethiol [**37**], which was detected in all three reaction mixtures, was also probably formed from the thermal degradation of cysteine[28].

These furan- and thiophenethiols have been found in the volatiles from different systems and some have been reported as volatile compounds in cooked meat. 2-Methyl-3-furanthiol [**19**] is considered to play an important role in the flavour of meat[15,16]. Moreover, it has been identified, together with 2-furylmethanethiol, as a high aroma value compound in chicken and bovine broths[29]. 2- or 3-Thiophenethiol [**37**] and 2-methyl-3-thiophenethiol [**50**] have been recently reported in pork flavour[30]. 2-Methyl-3-furanthiol [**19**], 2-furylmethanethiol [**29**] and 2-methyl-3-thiophenethiol [**50**] have been detected in various meat model systems, such as 5'-IMP/cysteine[10,13], ribose/cysteine[4], cystine/thiamine/glutamate/ascorbic acid[30,31]. Also [**19**] has been reported together with 2-methyl-4,5-dihydro-3-furanthiol [**36**] and 2-methyl-3-thiophenethiol [**50**], in the volatiles of thermally degraded thiamine[32].

The presence of thiol substituted furans and thiophenes, such as compounds (**19**, **29**, **36**, **37**, **50**, **54)**, gave rise to a number of important disulphides in the reaction mixtures. Those identified were: 2-methyl-3-furyl 1-methyl-2-oxopropyl disulphide [**87**], bis(2-methyl-3-furyl) disulphide [**88**], 2-methyl-3-furyl 1-ethyl-2-oxopropyl disulphide [**89**], 2-methyl-3-furyl 1-methyl-2-oxobutyl disulphide [**90**], 2-methyl-3-furyl 2-furylmethyl disulphide [**91**], bis(2-furylmethyl) disulphide [**92**], 2-methyl-3-furyl 2- or 3-thienyl disulphide [**93**], 2- or 3-thienyl 1-methyl-2-oxobutyl disulphide [**94**], 2-methyl-3-furyl 2-methyl-3-thienyl disulphide [**95**], 2-furylmethyl 2- or 3-thienyl disulphide [**96**], bis(2- or 3-thienyl) disulphide [**97**] and 2-methyl-3-thienyl 2- or 3-thienyl disulphide [**98**]. In the reaction mixture at pH 3.0, all of these 12 compounds were formed. Nevertheless four were not detected at pH 4.5 and none were found at pH 6.0.

The sulphur-substituted furans and thiophenes are likely to be very important in cooked meat aromas. Disulphides such as compounds **87**, **89**, **90**, **91**, **92**, **94**, **96**, **97**, **98** have been reported previously in different Maillard systems which involve a reducing sugar and a sulphur source, such as ribose or hydroxymethyl furanone (HMF) and cysteine or hydrogen sulphide (H_2S)[6,7,10,13,32]. The presence of compounds **88**, **91**, **92**, **93** and **95** in cooked meat has been reported by Farmer & Patterson[33] and Mottram & Madruga[16,17]. Compounds **88** and **95** have been identified in systems involving thiamine, and **87** and **95** in yeast extracts[34]. This is the first time that compounds **87**, **89**, **90**, **93**, **94**, **95**, **97**, **98** have been reported in a model system using 5'-IMP/cysteine.

Many thiophenes were detected in all three systems, the formation of alkylthiophenes [**10, 20**] and formyl thiophenes [**22, 43, 56, 59, 60, 70, 76**] were not pH dependent; however, thiophenes with acetyl or propionyl branches [**55, 63, 66, 68**] were detected only at pH 3.0. The four thiophenones identified [**33, 38, 40, 49**] were also clearly formed only at lower pH, with the exception of 4,5-dihydro-2-methyl-3(2H)-thiophenone [**40**], which appeared to be pH independent. Among the sulphur-containing compounds, thiophenes constitute a class which has been reported in volatiles from a number of foods[35], and they have been suggested as being responsible for the mild sulphurous odour of cooked meat[24]. The main routes for their formation involve the reaction of furfural and furanones with hydrogen sulphide[26, 27] or the condensation of mercaptoacetaldehyde with α,β-unsaturated aldehydes[5,27].

Several di-, tri- and tetrasulphur-containing five- or six-membered heterocyclic rings were identified in 5'-IMP/cysteine mixtures. Four dithiolanones were found in the reactions under acid conditions: 3-methyl-1,2-dithiolan-4-one [**52**], (*E*or*Z*) 3,5-dimethyl-1,2-dithiolan-4-one [**57, 58**] and 3-ethyl-1,2-dithiolan-4-one [**67**]. One trithiolane [**61**] together with two isomers (*E*or*Z*) 3,5-dimethyl-1,2,4,trithiolane [**62, 65**] were identified in reactions at all pHs. Dithiolanones are probably formed from the reaction between breakdown products of 5'-IMP and cysteine. Trithiolanes are likely to be formed from thermal degradation of cysteine[28]. Trithiolanes can be generated from the reaction of aldehydes/H_2S[27].

Dithianes, trithianes and tetrathianes, which are heterocyclic compounds with two, three and four sulphur atoms in six-membered rings, were also formed in these systems. Formation of dithianones and dithianes was favoured by acid pH; however more trithianes /tetrathianes were found at high pH. Trithianes can be formed from the reaction between saturated aldehydes, such as acetaldehyde, and hydrogen sulphide[27]. In the 5'-IMP+cysteine model systems the thermal degradation of cysteine to give acetaldehyde and hydrogen sulphide could be a route for their formation[28].

Garbusov *et al.*[36] reported 1,3- and 1,4-dithianes from volatiles of boiled beef. 3-Methyl-1,2,4-trithiane [**77**] has been identified in commercial beef extract[6] and in cysteine/ribose model systems[4,5]. The compound 2,4,6-trimethyl-1,3,5-trithiane [**86**] was reported in pressure-cooked beef by Wilson *et al.*[38], also in cooked chicken flavour[27]. 3-Methyl-1,2-dithian-4-one [**73**] and 3,5(or 6)-dimethyl-1,2-dithian-4-one [**71**] were found in model systems containing cysteine, ribose and phospholipid[4]; and in glutamate, ascorbate, thiamine and cysteine[39]. 3-Methyl-1,2-dithian-4-one [**52**] has been identified in different meat samples and in yeast extract[30,34,40].

Eight bicyclic compounds were found in these studies, *i.e.* 2,3-dihydro-6-methylthieno[2,3c]furan [**72**], thienothiophene [**78**], dihydrothienothiophene [**79**], methylthienothiophene [**80**, **83**], methyldihydrothienothiophene [**82**, **84**] and 2-ethylthienothiophene [**85**]. The 2,3-dihydro-6-methylthieno[2,3c]furan (kahweofuran) [**72**] is known as an important constituent of coffee volatiles. A number of alkylthienothiophenes were reported in the volatile components of ribose/cysteine model systems[4].

Except for thiazoles, there were no other nitrogen-containing volatile compounds identified from the 5'-IMP/cysteine at any of the three pHs. Absence of pyrazines at low pH was probably because the carbonyl-amino interaction is not favourable under acidic pH. Lack of pyrazine was noted by Zhang & Ho[10] in a 5'-IMP/cysteine mixture at pH 2.3 and Whitfield *et al.*[41] found that concentrations of nitrogen-containing heterocyclic compounds were reduced in systems containing cysteine and ribose, compared with other amino acids. Five thiazoles, including a series of acylthiazoles [**16, 42, 44, 53**] and a benzothiazole [**75**], were generated in the 5'-IMP/cysteine mixtures. Their formation does not seem to be highly influenced by changes in pH. The acylthiazoles have been reported in a model system containing cysteine and ribose[4,5]. Compound **75** was not reported in the volatiles of the ribose/cysteine system, however it has been found among the volatiles of roasted, fried and pressure cooked beef[42]. According to Vernin & Parkanyi[27], the thermal degradation of cysteine and cystine, either alone or in the presence of reducing sugars such as ribose, is a source of thiazoles, also they can be formed by heat-degradation of thiamine.

The breakdown of 5'-IMP appears to be the main source of furans, furfurals and furanones found in the reaction mixtures. Four alkylfurans containing chains of C1-C5 [**18, 24, 41, 45**], together with two furylketones [**34, 51**] and two acylfurans [**27, 39**] were formed. Another group of furans identified were furfural [**15**] and its derivatives [**30, 32, 48, 64**] and 4-hydroxy-5-methyl-3(2H)-furanone [**21**]. These compounds were highly influenced by pH changes. There was a clear tendency for many more furans being formed at low pH. Only 2-butylfuran [**24**] and 2-furfural [**15**] were detected in the reaction mixture at pH 6.0. One exception was found for 2,4-dimethylfuran [**18**] which was not detected at pH 4.5 and 3.0, although this compound may not have been fully captured during the headspace analysis due to its low boiling point and consequently to its high volatility. 4-Hydroxy-5-methyl-3(2H)-furanone [**21**] was found only in the reaction mixture at pH 4.5. It may have been formed at pH 3.0 but, in the presence of H_2S, this compound could have been converted into other volatiles. According to Mottram & Leseigneur[8], concentrations of furans/furfural/ furanones dropped markedly as the pH increased in model systems containing ribose and aminoacids.

2-Acetylfuran [**27**] and 1-(2-furyl)-2-propanone [**34**] have been found as products of the reaction between cysteine and ribose[5] and in fried beef[35]. Most of the other furans [**15, 24, 27, 30, 34, 41, 45, 48, 51, 64**] have been reported in similar model systems[4]. 4-Hydroxy-5-methyl-3(2H)-furanone [**21**] has been identified in meat by Tonsbeek *et al.*[43] who proposed it was formed from a ribonucleotide via a ribose-5'-phosphate.

The presence of ketones in the 5'-IMP/cysteine system can be explained by Maillard-type reactions and Strecker degradation of cysteine, which can lead to the formation of short chain ketones [**3, 6, 7, 8, 9, 11, 17**]. However the formation of 2-hexanone [**12**], 2-heptanone [**23**] and 3-heptanone [**25**] cannot be explained by these routes/mechanisms; they may have been formed by the condensation reactions between other carbonyl products. Ketones were more abundant in 5'-IMP/cysteine system at pH 6.0, which could result from the fact that enolisation of Amadori products in the Maillard reaction is pH dependent and dicarbonyl formation is more pronounced at alkaline conditions. Alternatively, at lower pH they may be lost due to further reaction with cysteine or hydrogen sulphide. In general, dicarbonyls like 2,3-pentanedione [**8**] and 2,3-butanedione are important intermediates in the formation of other volatile products, as has been discussed in this text. They can react with hydrogen sulphide leading to the formation of mercapto ketones which can produce important meat-like volatiles. 2-Methylcyclopentanone [**17**] was identified in the volatiles of fresh cooked ground beef and freeze-dried defatted rehydrated beef. It was also reported to contribute to increase meatiness in some flavour isolates[18].

Three mercaptoketones [**14, 26, 28**] were formed mainly at low pH (4.5 and 3.0). Only 3-mercapto-2-butanone [**14**] clearly appeared in the reaction mixture at pH 6.0, although 3-mercapto-2-pentanone [**26**] was detected as a trace peak at this pH. The mercaptoketones were probably formed from the reaction of the corresponding alkanedione (breakdown products of 5'-IMP) with hydrogen sulphide, i.e. 2,3-butanedione/H_2S or 2,3-pentanedione/ H_2S. Some mercaptoketones, such as 3-mercapto-2-pentanone, 1-mercapto-3-pentanone, 1-mercapto-2-propanone have been identified in meat aroma model systems[4, 10]. 3-Mercapto-2-pentanone has been reported in the volatiles of boiled meat and chicken broth[29].

Conclusion

The results have shown that the reaction between inosine-5'-monophosphate (5'-IMP) and cysteine led mainly to the formation of heterocyclic compounds (seventy-six volatiles). Many of these were sulphur-containing volatiles, such as disulphides and thiophenes, other compounds identified were thiazoles, furans, mercaptoketones, bicyclic and cyclic sulphur-compounds. A clear tendency was observed for some classes of compounds to be formed more at higher or lower pH, for instance, sulphur-containing compounds, such as sulphur-substituted furans and mercaptoketones, as well as alkylfurans, were more readily formed at lower pH, while pyrazines were inhibited by acidic conditions. Other heterocyclic compounds like thiazoles and trithiolanes were not affected by pH changes.

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