STUDIES ON SOME PRECURSORS INVOLVED IN MEAT FLAVOUR FORMATION[1]

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SUMMARY

The effect of some precursors on the formation of meat flavour during heating has been investigated. A comparison of the influence of three different precursors, inosine-5'-monophosphate (5'-IMP), cysteine and thiamine, added to the meat systems, showed that formation of certain heterocyclic compounds, like sulfur-containing furans, dithiolanones and thiophenes, was significantly affected by changes in the concentration of precursors. However, aliphatic compounds, such as hydrocarbons, alcohols and ketones were not changed by these additions. Inosine-5'-monophosphate was established to be more effective than cysteine or thiamine in the formation of some "meaty" volatiles, i.e. the furanthiols, when its concentration was increased 10 times in raw meat.

Key words: Meat, flavour, precursors, 5'-IMP, cysteine, thiamine.

RESUMO

ESTUDOS DE ALGUNS PRECURSORES ENVOLVIDOS NA FORMAÇÃO DO AROMA CÁRNEO. O efeito de alguns precursores na formação do aroma cárneo durante o aquecimento foi investigado. Estudos comparativos da influencia de três diferentes precursores, inosina-5'-monofosfato (5'-IMP), cisteína e tiamina, adicionados aos sistemas cárneos, mostrou que a formação de certos compostos heterocíclicos, como furanos sulfurados, ditiolanonas e tiofenos, foi significativamente afetada pelas mudanças de concentração dos precursores. Entretanto, compostos alifáticos, tais como: hidrocarbonetos, alcoois e cetonas não foram afetados por estas adições. Inosina-5'-monofosfato apresentou-se como o precursor mais eficiente na formação de alguns voláteis de aroma cárneo, como por exemplo os furanos sulfurados, quando aumentou-se em 10 vezes sua concentração na carne crua.

1 — INTRODUCTION

Flavour is one of the most important attributes of cooked meat, it results from reactions involving different precursors during cooking. Cysteine, thiamine and ribonucleotides (particularly 5'-IMP) are believed to be important meat flavour precursors. A significant amount of research on volatile compounds which contribute to meat flavour has been carried out and the identities of some important compounds has been established mainly from heated model systems containing mixtures of these precursors (6, 12, 19, 26, 27, 36, 37, 38). In search for compounds with meat-like aroma, furans and thiophenes with a thiol in the 3-position have been identified as the most important (2). They were formed mainly in heated model systems containing hydrogen sulfide or cysteine and pentoses or other sources of carbonyl compounds (4, 19, 36, 38), also recently these sulfur compounds have been reported in meat itself (5, 8, 18, 21, 22, 35). 2-Methyl-3-furanthiol and its corresponding disulfide - bis(2-methyl-3-furyl) disulfide - were found to be very powerful volatiles with meat-like characteristics and exceptionally low odour threshold values (1, 2, 8) and their formation in model systems appeared to be influenced by different factors as pH, temperature, as well as the nature and concentrations of reactants (4, 11, 21, 22, 26, 28, 29)

This paper reports studies undertaken to establish the effect of addition of some meat flavour precursors, i.e. 5'-IMP, cysteine and thiamine, on the formation of volatile compounds in cooked beef. Inosine-5'-monophosphate was chosen because it the principal source of pentose sugar in muscles, which accumulates in post-slaughter muscle through the hydrolysis of adenosine triphosphate (20). Cysteine appears to be indispensable for the development of the characteristic flavour of meat. Thiamine has been recognised for some time as an important source of meaty aroma compounds (14, 15).

2 — MATERIAL AND METHODS

2.1 - Cooked meat

Portions (100g) of minced beef *M. Psoas major*, obtained from a local meat supplier, were chopped in small pieces and mixed in a laboratory blender with 12 ml water containing different meat flavour precursors. 5'-IMP (2.7g) was added to one minced meat sample, cysteine (39.3mg) was added to another portion and thiamine (1.0mg) was added to a third sample. This resulted in an increase of approximately ten times in the concentration of these precursors compared with normal meat. The addition of 5'-IMP resulted in a drop in pH of 1.1 pH units; therefore the pH was adjusted, before heating, to 5.6 by adding 1 M sodium hydroxide. A meat blank was prepared in which the addition of precursors was omitted. After adjustment, samples were left overnight in a refrigerator. They were heated in glass bottles in an autoclave at 140°C for 30min.

2.2 - Methods

2.2.1 - Headspace analysis of volatiles

Headspace volatiles from the cooked meat were analyzed by headspace concentration followed by GC or GC-MS. The meat was transferred to a 250ml conical flask fitted with 30mm screw joints to take a sliding joint with PTFE seal and Dreschel head. The volatiles were collected in a glass-lined stainless steel trap (155mm long x 0.75mm id) packed with Tenax-GC (SGE Ltd.). The volatiles were swept on to the adsorbent in the trap using a flow of oxygen-free nitrogen (40 ml/min); the collection was continued for 2h. The sample was maintained at 60°C, in a water-bath with cons-

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tant agitation, 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 sample.

2.2.2 - Gas Chromatography

After collection, the volatiles were thermally desorbed, using a modified injector port, directly onto the front of a DB-5 fused silica capillary column (30 m x 0.32 mm i.d.) (JW Scientific Inc) in the oven of a Hewlett Packard HP5890 Gas Chromatograph. The oven was held initially at 0°C for 5 min while the volatiles were desorbed from the Tenax trap (held at 250°C in the modified injector). The column temperature pH after the removal of the coolant, was rapidly increased to 60°C and after this, it was maintained at 60°C for 5 min. Then it was programmed at 4°C/min to the final temperature (250°C) where it was held for a further 20 min. At the end of the column, the effluent was split 1:1 to a flame ionization detector (FID) and an odour port. Helium at 2mL/min was used as a carrier gas. A solution containing C_6-C_{20} n-alkanes was chromatographed before each cooked meat containing added precursor was analyzed, to allow calculation of linear retention indices (LRI) for each sample component. For each meat sample the aroma of the GC effluent was assessed separately by four individuals, experienced in aroma evaluation, who provided a description for each aroma detected.

2.2.3 - Gas Chromatography-Mass Spectrometry (GC-MS)

The cooked meat samples were also analyzed by GC-MS using a Hewlett Packard HP5988A Mass Spectrometer fitted with an HP 5980 GC and a HP Chemstation data system. The GC column and conditions used were the same as those described above. The following conditions were used for the mass spectrometer: source temperature = 250°C; ionising voltage = 70 eV; scan range from m/z 29-290, with one scan per second. Quantization of volatiles was based on peak area integration of the GC-MS chromatograms using 1,2-diclorobenzene as internal standard. This compound in ethanolic solution (1ml containing 65ng) was added to the trap immediately after the collection.

2.2.4 - Assessment of samples after heating

In addition to the odour port analysis, samples of cooked meat were subjected to a simple assessment of the overall odour, after cooking and immediately prior to headspace collection. They were transferred to conical flasks which were wrapped with foil to avoid any visual bias; and then their odour was assessed by four individuals.

3 — RESULTS AND DISCUSSION

Volatile components identified in the headspace of four different systems: meat with added 5'-IMP, meat with added cysteine, meat with added thiamine and the meat blank are listed by classes of compounds in *Table 1*. The 126 volatiles presented were those which gave significant peaks in the gas chromatograms, together with minor components of possible odour significance, e.g. compounds which were described as having a meat-like aroma or a similar description.

TABLE 1. Approximate concentrations[1] (ng/100g meat) of compounds identified in the headspace volatiles from cooked meat with added 5'-IMP, cysteine or thiamine. Each value is the mean of three analysis and the standard deviation is given in parentheses.

		Amounts[2]			
Compound	LRI	Meat + 5'-IMP	Meat + THI	Meat + CYS	Meat BLK
HYDROCARBONS					
hexane	603	36 (2.9)	34 (1.8)	nd	11 (2.8)
niethylcyclopentane	624	3 (0.5)	6 (0.8)	nd	nd
cyclohexane	638	34 (0.7)	37 (1.4)	36 (2.1)	25 (5.9)
heptane	701	8 (0.7)	3 (0.6)	7 (1.4)	nd
a methylbenzene	774	8 (3.3)	11 (0.8)	7 (1.6)	7 (1.9)
2-pentene	785	nd	nd	nd	56 (2.2)
1,2 or 1,4-dimethylbenzene	855	13 (0.2)	14 (0.9)	15 (2.3)	11 (6.8)
ethylbenzene	861	4 (1.4)	4 (2.7)	4 (2.1)	8 (0.9)
1,3-nonadiene	928	47 (0.4)	46 (2.2)	46 (1.7)	35 (3.3)
undecane	1100	nd	nd	158(23)	142 (17.8)
dodecane	1200	74 (0.6)	76 (1.5)	73 (1.9)	72 (11.9)
tridecane	1300	73 (11)	77 (19)	47 (18)	62 (5.9)
tetradecane	1400	13 (2.1)	12 (0.4)	10 (7.1)	18 (5.1)
pentadecane	1500	68 (26)	63(44)	89 (42)	69 (36)
heptadecane	1700	20 (8)	27 (10)	39 (4.9)	18 (5.3)
octadecane	1800	67 (13.9)	53 (19)	29 (11.6)	43 (8.8)
3,7,11,15-tetramethyl-2-hexadecene	1850	13 (10.7)	13 (1.7)	10 (4.9)	12 (1.2)
nonadecane	1900	4 (3.9)	2 (0.5)	5 (3)	4 (0.9)
limonene	1033	282 (36.4)	253(33)	194 (50.7)	168 (46.2)
naphtalene	1184	6 (4.1)	4 (1.2)	13 (6.9)	13 (8.2)
ALDEHYDES					
3-methylbutanal	640	24 (15)	25 (5.4)	74 (36)	22 (3.1)
2-methylbutanal	667	19 (14.8)	24 (4.8)	12 (10.7)	12 (8.3)
pentanal	709	20 (20.9)	5 (1.2)	nd	nd
hexanal	801	86 (46.9)	128 (36)	159 (10.9)	114 (67.7)
heptanal	903	116 (73.1)	126 (53)	557 (65.8)	322 (216.7)
benzaldehyde	961	1708 (405)	1180(28)	1393 (33)	1506 (295.5)
octanal	1006	3248 (96.3)	3733(59)	3846 (69)	3363 (93.2)
2-methylbenzaldehyde	1048	133 (10.3)	130 (43)	162 (30.4)	135 (18.4)
(E)-2-octenal	1063	215 (17.5)	230 (11)	267 (36.8)	220 (12.1)
nonanal	1109	5328(173)	5776(98)	5829 (99)	5611 (219.9)
(E)-2-nonenal	1160	124 (6.7)	161 (14)	209 (52.3)	124 (91.1)
decanal	1204	119 (7.1)	218 (29)	245 (20.4)	169 (47.3)
undecanal	1306	7 (2.8)	7 (1.9)	15 (7)	10 (5.9)
(E,E)-2,4-decadienal	1316	55 (9.9)	59 (2.2)	72 (6)	58 (36)
(E)-2-undecenal	1363	11 (2.5)	34 (2.4)	50 (16.1)	71 (8.8)
dodecanal	1408	39 (4.2)	37 (1.9)	43 (5.7)	36 (4.6)
tridecanal	1510	110 (43.2)	112 (9.9)	137 (26.2)	115 (40.2)
tetradecanal	1611	70 (13.5)	96 (31.2)	76 (13.8)	89 (20.9)
pentadecanal	1712	343 (24.7)	317 (16)	350 (25.6)	341 (24.1)
hexadecanal	1817	3224 (245)	2965(51)	3348(306	2925 (265.1)
KETONES					
2-pentanone	712	nd	nd	nd	4 (1.8)
2,3-pentanedione	726	44 (13.7)	19 (2.4)	23 (10.5)	16 (13.3)
3-hydroxy-2-butanone	744	52 (4.1)	13 (2)	22 (4.6)	13 (3.2)
2,4-pentanedione	786	15 (4.9)	nd	10 (1.1)	nd
2-hexanone	794	63 (10)	46 (3.6)	64 (8.5)	31 (23.2)
2-heptanone	890	441 (46.3)	398 (12)	434 (98.3)	414 (16)
(E) 3-octen-2-one	1010	nd	nd	nd	99 (20)
2-nonanone	1089	132 (40.8)	106 (16)	210 (25.7)	129 (19)
2-decanone	1193	131 (15.4)	128 (57)	176 (35.4)	144 (34.1)

2-dodecanone	1393	23 (16)	22 (14)	22 (11.5)	28 (1.2)
2-tridecanone	1495	59 (5.3)	64 (12.9)	60 (7.2)	54 (42)
2-hexadecanone	1799	211 (66.3)	146 (21)	212 (12.2)	141 (33.9)
ALCOHOLS					
1-hexanol	871	139 (18.3)	168 (26)	155 (65.9)	149 (23)
1-heptanol	977	232 (28.9)	246 (36)	316 (89.7)	266 (37.4)
1-octen-3-ol	985	1390(125)	1383 (81)	1463 (123	1378 (118.1)
1-octanol	1080	717 (140.2)	732 (69)	770 (26.9)	727 (32.9)
1-nonanol	1073	60 (17.5)	57 (15.6)	51 (19.l)	54 (17.9)
1-decanol	1261	213 (45)	300 (41)	285 (34.1)	241 (66.8)
1-dodecanol	1472	134 (19.6)	124 (18)	115 (14.7)	111 (29.6)
1-tridecanol	1579	60 (23.5)	46 (7.5)	62 (15.9)	44 (2.9)
1-tetradecanol	1676	229(134)	219(48)	229 (76.4)	238(47)
ALKYL SULFIDES					
dimethyl disulfide	763	13 (4.9)	14 (5.1)	22 (2.9)	12 (1.7)
3-mercapto-2-butanone	813	17 (1.7)	6 (0.6)	12 (0.9)	tr
3-(methylthio)-propanal	907	45 (10.4)	9 (2.6)	30 (2.9)	tr
2-mercapto-3-pentanone	908	104 (14)	39 (9.4)	30 (12.7)	tr
dimethyl trisulfide	967	286 (19.2)	269(27)	310 (27.9)	267 (40.8)
methyl pentyl disulfide	1132	10 (4.1)	9 (1.6)	15 (1.5)	9 (2.9)
dimethyl tetrasulfide	1215	21 (3.7)	19 (2.5)	24 (2.6)	18 (7.7)
dipropyl trisulfide	1348	7 (1.6)	8 (0.8)	9 (2.3)	5 (1.5)
S-SUBSTITUTED FURANS					
2-methyl-3-furanthiol	873	13 (5.9)	tr	3 (1.0)	tr
2-furylmethanethiol	913	250 (21.5)	207(38)	124(24)	142(35)
2-methyl-3-furyl methyl disulfide	1175	23 (6.1)	19 (4.9)	13 (3.3)	tr
2-furylmethyl methyl disulfide	1220	74 (12.9)	19 (3.6)	23 (14.9)	21 (15.7)
2-methyl-3-furyl methyl trisulfide	1392	3 (1.3)	4 (0.2)	2 (1.3)	tr
2-furylmethyl methyl trisulfide	1451	55 (12.2)	19 (3.6)	9 (1.4)	30 (11.9)
1-(2-methyl-3-furyldithio)-2 -propanone	1466	tr	nd	tr	nd
bis(2-methyl-3-furyl) disulfide	1535	tr	tr	nd	nd
1-(2-furylmethyldithio)-2-propanone	1552	5 (2.9)	2 (0.8)	6 (0.4)	tr
2-(2-methyl-3-furyldithio)-3 - pentanone	1584	tr	nd	nd	nd
2-(2-furylmethyldithio)-3-butanone	1587	15 (1.9)	22 (1.7)	7 (0.4)	5 (0.3)
2-methyl-3-(2-furylmethyldithio) furan	1635	8 (1.9)	5 (0.5)	2 (0.2)	4 (1.4)
1-(2-furylmethyldithio)-2-butanone	1649	5 (2.2)	7 (1.9)	3 (2.7)	tr
2-(2-furylr-nethyldithio)-3-pentanone	1659	4 (2)	tr	tr	nd
bis(2-furylmethyl) disulfide	1687		20 (12.5)		30 (5.9)
bis(2-furylmethyl) trisulfide	1932	13 (8.2)	6 (0.9)	17 (1.6)	7 (1.9)
THIOPHENES AND THIOPHENONES					
2-methylthiophene	779	16 (6)	13 (1.2)	10 (6.1)	14 (3.3)
2-ethylthiophene	859	nd	nd	7 (4.4)	nd
2-formylthiophene	1002	191 (17.5)	7 (3.5)	602 (67)	2 (0.7)
2-acetylthiophene	1087	15 (2.3)	nd	59 (22)	nd
a formylmethylthiophene	1097	47 (12.3)	60 (4.6)	74 (7.3)	51 (3.6)
2-formyl-5-methylthiophene	1120	10 (0.9)	17 (6.9)	40 (12.8)	17 (11.7)
2-formyl-3-methylthiophene	1124	16 (5.2)	13 (3.7)	38 (39.1)	27 (7.4)
3-acetyl-2,5-dimethylthiophene	1267	6 (3.4)	3 (0.5)	2 (1.5)	tr
2-hexylthiophene	1278	nd	nd	5 (1.2)	tr
4,5-dihydro-3(2H)-thiophenone	950	42 (23.3)	44 (15)	41 (20.7)	52 (30.8)
4,5-dihydro-5-methyl-3(2H)- thiophenone	980	204 (128)	140 (39)	130 (70)	196 (85)
4,5-dihydro-2-methyl-3(2H)- thiophenone	987	128 (24.2)	77 (24)	88 (15.2)	73 (8.8)
DITHIOLANONES AND DITHIANONES					
3-methyl-l,2-dithiolan-4-one	1072	45 (21.9)	42 (3.8)	83 (45.4)	43 (9.4)
(EorZ) 3,5-dimethyl-1,2-dithiolan-4-one	1104	33 (7.8)	nd	58 (23.4)	nd
1,2-dithian-4-one	1165	33 (ll.8)	30 (13)	48 (31.5)	24 (7.1)
THIAZOLES					
4,5-dimethylthiazole	933	nd	nd	25 (7.1)	38 (1)
2-acetylthiazole	1020	227 (41)	194 (23)	269 (41)	165 (36)
2,4 or 2,5-diethylthiazole	1117	20 (12.1)	16 (6.2)	13 (7.9)	nd
2,4-dimethyl-5-ethylthiazole	1121	20 (4.4)	nd	nd	nd
BICYCLIC COMPOUNDS					
a thienothiophene	1057	5 (2.9)	4 (1.6)	16 (9.2)	tr
2,3-dihydro-6-methylthieno[2,3c]furan	1188	55 (12.8)	52 (8.8)	56 (29)	44 (17.8)
a methyldihydrothienothiophene	1363	11 (2.5)	24 (2.4)	19 (4.9)	tr
FURANS AND FURANONES					
2-ethylfuran	707	9 (2.4)	nd	16 (7.4)	24 (9.4)
2-pentylfuran	994	1038 (131)	1002 (2)	1192 (74)	917 (63)
2-hexylfuran	1089	73 (40.8)	36 (16)	210 (26)	112 (40)
2-propionylfuran	1013	18 (8.9)	29 (8.9)	27 (15.9)	38 (2.5)
dihydro-3,5-dimethyl-3(2H)- furanone	781	nd	nd	nd	13 (1.7)
4,5-dihydro-2-methyl-3(2H)- furanone	806	98 (12.4)	62 (12)	61 (10)	51 (8.4)
2-furfural	828	36 (17.7)	12 (7.2)	18 (6.4)	50 (26.5)
2-furanmethanol	862	1 (0. 9)	3 (1.1)	3 (0.8)	3 (0.8)
1-(5-methyl-2-furyl)-2-propanone	1054	10 (3.5)	9 (3.3)	13 (8)	13 (9.2)
1-(5-methyl-2-furyl)-3-butanone	1086	30 (15.4)	29 (3.4)	54 (27.5)	24 (17.2)
PYRAZINES					
methylpyrazine	820	51 (20.3)	19 (1.9)	41 (21.4)	26 (8.1)
2,6 or 2,5-dimethylpyrazine	914	176 (10.4)	130 (26)	201 (49)	143 (46)
ethylpyrazine	920	167 (27.4)	68 (2.7)	nd	nd
2,3-dimethylpyrazine	921	11 (5.8)	28 (2.8)	nd	65 (5.5)
vinylpyrazine	935	18 (11)	5 (1.7)	26 (3.7)	nd
2-ethyl-(5 or 69-methylpyrazine	997	22 (12.3)	24 (12)	nd	29 (8.3)
2-ethyl-3,6-dimethylpyrazine	1085	157 (22.3)	130 (46)	120 (29)	121 (38)
2,3-diethyl-5-methylpyrazine	1158	110 (10.5)	97 (28)	102 (31)	91 (3.6)

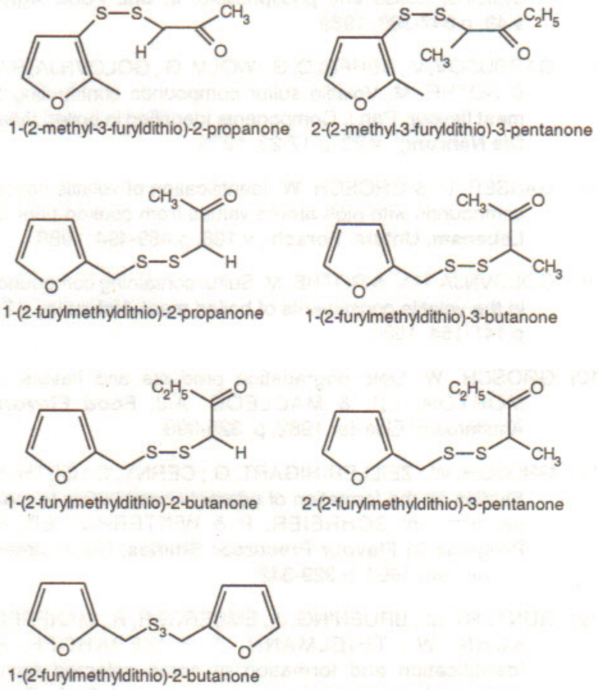
1 concentrations obtained by comparing GC/MS peak areas with the area of 65 ng dichlorobenzene added to Tenax trap as internal standard

2 tr, trace (.2 ng/100g meat); nd, not detected; + present in significant amounts but quantitation confunded by large adjacent peak

Ninety-five compounds were positively identified by comparison of their mass spectra and LRIs with authentic compounds. Identifications based on comparison with published mass spectra, have been proposed for 31 other compounds where reference LRI were unavailable. The approximate concentrations were obtained by comparison of GC/MS peak areas in the total ion chromatogram (TIC) with the wazzu area of 65 ng 1,2-dichlorobenzene (internal standard). Values were averaged over at least three replicates for each system. It should be emphasised that the method used for quantitation only gives an indication of the approximate quantity present in collected headspace volatiles. More accurate quantitation using calibration standards for all compounds was not practical, neither was it necessary, since the aim of the experiment was to compare the effect of the different treatments.

Aliphatic components (in a total of 61 compounds) were more abundant than heterocyclic products (56 compounds), and there were a small number, 9 aromatic compounds. The aliphatic compounds comprised: 13 hydrocarbons, 18 aldehydes, 13 ketones, 9 alcohols, 6 alkanethiols and 2 mercaptoketones. The group of heterocyclic compounds encompassed: 16 sulfur-substituted furans, 12 thiophenes, 2 dithiolanones, 1 dithianone, 4 thiazoles, 3 bicyclic compounds, 10 other furans and 8 pyrazines. The majority of these compounds have been identified previously in heated beef aromas (21, 22), their precursors and possible pathways involved in their formation have been recently reviewed by Mottram (23, 24, 25).

Among the 16 sulfur-substituted furans found in the meat systems seven are reported here for the first time in cooked beef aroma. These compounds are 1-(2-methyl-3-furyldithio)-2-propanone, 1-(2-furylmethyldithio)-2-propanone, 2-(2-methyl-3-furyldithio)-3-pentanone, 2-(2-furylmethyldithio)-3-butanone, 1-(2-furylmethyl dithio)-2-butanone, 2-(2-furylmethyldithio)-3-pentanone and bis(2-furylmethyl) trisulfide. Some of these compounds have been recently synthesized from the reactions of alkanediones with hydrogen sulfide and furanthiols (27). The structures of these compounds are shown in *Figure 1*.


1-(2-methyl-3-furyldithio)-2-propanone 2-(2-methyl-3-furyldithio)-3-pentanone

1-(2-furylmethyldithio)-2-propanone 1-(2-furylmethyldithio)-3-butanone

1-(2-furylmethyldithio)-2-butanone 2-(2-furylmethyldithio)-3-pentanone

1-(2-furylmethyldithio)-2-butanone

FIGURE 1. New sulfur-substituted furans identified in cooked meat volatiles.

Aliphatic hydrocarbons, aldehydes, alcohols and ketones are known to be major volatiles products from heated meat and therefore it was not surprising that they were significant among the volatiles. Aldehydes constituted the major class of volatiles, contributing with nearly 65% of the total GC-MS peak area. Nonanal was the most abundant compound found.

Increasing the concentrations of the precursors in raw meat affected principally the formation of some heterocyclic compounds, while the formation of hydrocarbons, ketones, alcohols was not clearly affected by addition of 5'-IMP, cysteine or thiamine.

Sulfur-substituted furans, mainly those possessing the 2-methyl-3-furyl moiety, were highly affected by addition of precursors. A number of these furans were either not detected or were only found at very low concentrations in meat without added precursors. Also an important impact meaty compound, bis (2-methyl-3-furyl) disulfide (8), was only detected in meat with added precursors. In general the mechanism of formation of 2-methyl-3-furanthiol could involve the initial formation of 4-hydroxy-5-methyl-3(2H)-furanone and dicarbonyls (from 5'-IMP) and their subsequent reaction with hydrogen sulfide (from cysteine or thiamine) (33). On the other hand, furanthiols containing the 2-furylmethyl moiety, such as 2-furylmethanethiol, bis(2-furylmethyl) disulfide, 2-furylmethyl trisulfide, 2-(2-furylmethyldithio)-3-butanone and bis(2-furylmethyl) trisulfide, were formed in all systems, including meat blank, and the amounts formed were generally unaffected by the addition of precursors. It can indicate that they were not derived directly from 5'-IMP or cysteine or thiamine.

Moreover, it was interesting to observe that compounds containing the 2-methyl-3-furyl moiety possessed meaty aromas during odour port analysis, but were more sulfurous at higher concentrations, whereas the 2-furylmethyl group gave more roast and nut-like aromas to compounds containing this moiety, these results agree with previous observations on the aroma of sulfur-substituted furans (2).

In these meat systems availability of hydrogen sulfide from cysteine and sugar degradation products (hydroxyfuranones) from 5'-IMP is likely to be the reason why some more cyclic sulfur compounds (thiophenes, dithiolanones and dithianones) were formed in meat system with added 5'-IMP or cysteine. No clear tendency was observed for the formation of thiazoles, pyrazines and alkyl furans in heated meat regarding the increase in concentration of meat precursors. Nevertheless, considerably less 2-furfural was obtained from meat with added precursors, a reason could be the fact that it is a breakdown product of pentose, which could act as intermediate product for the formation of other heterocyclic compounds, and may have been lost by further reaction.

Among the aliphatic compounds identified in cooked meat, the alkyl sulfides were highly affected by addition of precursors to the meat. These compounds were fairly formed in systems with added 5'-IMP, cysteine or thiamine, although the addition of cysteine to meat, in general results in the highest levels of them. As cysteine and thiamine break down to hydrogen sulfide, it will contribute to increase the levels of sulfides in these systems (13, 14, 32). 5'-IMP probably favoured the formation of sulfides throughout the production of higher levels of carbonyls, which can be formed from sugar sources (16). Aliphatic polysulfides, such as dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide have been identified repeatedly in the volatiles of cooked meat (7, 9, 17,23).

Two identified mercaptoketones, 3-mercapto-2-butanone and 2-mercapto-3-pentanone, were reported for the first time in cooked meat. They were present at highest concentration in meat with added 5'-IMP, but were detected only in trace amounts in meat blank. Reaction of carbonyls groups with hydrogen sulfide provides a rote to mercaptoketone formation.

The concentrations of hydrocarbons, ketones and alcohols in cooked meat systems did not show obvious trends for any of the four systems. The origin of these aliphatic compounds in meat volatiles can be explained from the thermal degradation of lipids present in lean meat, involving the thermal oxidation of the long-chain fatty acids (10). These reactions are not supposed to be affected by addition of 5'-IMP, cysteine and thiamine. In addition, some aliphatic compounds deserve comments due to their importance in meat flavour. 3-Hydroxy-2-butanone which is a fission and intermediate product of the Maillard reaction, increased significantly when 5'-IMP or cysteine was added to meat, it would resulted from the thermal degradation of ribose from 5'-IMP source. 1-Octen-3-ol was fairly formed in all wazzu meat systems, specially in meat with added cysteine, which could be a result of the increase in the autoxidation process of fatty acid cause by cysteine. Because of its high concentration associated with its low odour threshold value (34) this compound would play an important part in the fatty characteristics of these meat systems. It was reported as one of the main headspace volatiles of cooked beef (30, 31).

An interesting trend was observed for the aldehydes, there was a tendency for large quantities of them to be formed in systems with added cysteine, though thiamine also increased their formation. The only explanation for the increase in the levels of aldehydes by the presence of cysteine could be the enhancement of the oxidation pathways responsible for their formation during the lipid oxidation. However, Farmer & Mottram (3) reported that addition of ribose and cysteine to model systems containing triglyceride or phospholipids reduced considerably the formation of aldehydes, it is difficult to explain the differences observed between the model and the meat systems. In meat with added cysteine, there may be insufficient sugar for the Maillard reaction intermediates to be formed in sufficient quantities to inhibit aldehyde concentrations.

The odour assessment of each system showed some variations, which were associated with some of the differences observed in the chemical classes of compounds. The aromas for all cooked meat systems were characterised by "beefy", "meaty", "boiled meat" , "roasted meat" and related terms, however meat with added 5'-IMP has the strongest meaty aroma, whereas meat blank had the weakest meaty aroma among the four systems. The strongest meaty aroma obtained in meat with added 5'-IMP could be explained by the larger amounts of some recognized meat-like compounds, such as sulfur-substituted furans.

4 — CONCLUSION

The present work has demonstrated that addition of 5'-IMP, cysteine or thiamine to raw meat caused different effects on the amounts of heterocyclic compounds identified. Some compounds such as sulfur-containing furans were mainly increased by the addition of 5'-IMP. Cysteine favoured the formation of thiophenes and dithiolanones. Alkyl sulfides and mercaptoketones were increased by increasing the concentrations of these three precursors.

5 — LITERATURE CITED

(1) BUTTERY, R. G.; HADDON, W. F.; SEIFERT, R. M.; TURNBAUGH, J. G. Thiamin odor and bis(2-methyl-3-furyl) disulphide. ***J. Agric. Food Chem.*** v.32, p.674-676 , 1984.

(2) EVERS, W. J.; HEINSOHN, H. H.; MAYERS, B. J.; SANDERSON, A. Furans substituted in the three position with sulfur. In: CHARALAMBOUS, G. & KATZ, I. ***Phenolic, Sulfur and Nitrogen Compounds in Food Flavors***, .Washington DC: American Chemical Society, 1976, p 184-193.

(3) FARMER, L.J. & MOTTRAM, D.S. Effect of cysteine and ribose on the volatile thermal degradation products of a triglyceride and three phospholipids. **J. Sci. Food Agric.**, v.60, p.489-497,1992.

(4) FARMER, L. J.; MOTTRAM, D. S. Recent studies on the formation of meat-like aroma compounds. In: BESSIERE, Y & THOMAS, A.F. **Flavour Science and Technology**, Chichester: Wiley, 1990, p 113-116.

(5) FARMER, L. J.; PATTERSON, R. L. S. Compounds contributing to meat flavour. **Food Chem.** v.40, p.201-205, 1991.

(6) FARMER, L. J.; MOTTRAM, D. S.; WHITFIELD, F. B. Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. **J. Sci. Food Agric.** v.49, p.347-368, 1989.

(7) GARBUSOV, V., REHFELD, G., WOLM, G., GOLOVNJA, R.V. & ROTHE, M. Volatile sulfur compounds contributing to meat flavour. Part I. Components identified in boiled meat. **Die Nahrung** , v.20, p.17-23, 1975.

(8) GASSER, U. & GROSCH, W. Identification of volatile flavour compounds with high aroma values from cooked beef. **Z. Lebensm. Unters. Forsch.**, v.186, p.489-494, 1988.

(9) GOLOVNJA, R.V. & ROTHE, M. Sulfur containing compounds in the volatile constituents of boiled meat. **Nahrung,** v.24, p.141-154, 1980.

(10) GROSCH, W. Lipid degradation products and flavors. In MORTON, I.D. & MACLEOD, A.J. **Food Flavors**, Amsterdam: Elsevier,1982, p. 325-398.

(11) GROSCH, W.; ZEILLER-HIGART, G.; CERNY, C.; GUTH, H. Studies on the formation of odorants contributing to meat flavours. In: SCHREIER, P & WINTERHALTER, P. **Progress in Flavour Precursor Studies**, Carol Stream IL:. Allured, 1993, p 329-342.

(12) GUNTERT, M., BRUENING, J., EMBERGER, R., MANFRED, KUHN, W., THIELMANN, T. & WERKHOFF, P. Identification and formation of some selected flavor compounds in various meat model systems. **J. Agric. Food Chem**, v.38, p.2027-41, 1990.

(13) GUNTERT, M.; BERTRAM, H. J.; HOPP, R.; SILBERZAHN, W.; SOMMER, H.; WERKHOFF, P. Thermal generation of flavor compounds from thiamin and various amino acids. In: HOPP, R. & MORI, K. **Recent developments in flavor and fragrance chemistry**.Weinheim: VCH, 1993a, p 215-240.

(14) GUNTERT, M.; BERTRAM, H. J.; EMBERGER, R.; HOPP, R.; WERKHOFF, P. Some new aspects of the thermal generation of flavour compounds from thiamin. In: SCHREIER, P. & WINTWRHALTER, P. **Progress in Flavour Precursor Studies**, Carol Stream IL: Allured Publ. Co., 1993b, p 361-378.

(15) HARTMAN, G. J.; CARLIN, J. T.; SCHEIDE, J. D.; HO, C. T. Volatile compounds from the thermal degradation of thiamin at high and low moisture levels. **J. Agric. Food Chem**. v.32, p.1015-1018, 1984.

(16) HODGE, J.E. Origin of flavor in foods.:Nonenzymatic browning reactions. In: SCHULTZ, H.W.; DAY, E.A. & LIBBEY, L.M. **Chemistry and Physiology of Flavors,** Westport: AVI Publ., 1967, p. 465-491.

(17) MACLEOD, G.M. & SEYYEDAIN-ARDEBILI, M. Natural and simulated meat flavors (with particular reference to beef). **CRC Crit. Rev. Food Sci. Nutr.**, v.14, p. 309-437, 1981.

(18) MACLEOD, G. & AMES, J. M. 2-Methyl-3-(methylthio)furan: a meaty character impact aroma compound identified from cooked beef. **Chem. Ind.** *(London),* p. 175-176, 1986.

(19) MADRUGA, M.S. **Studies on some factors affecting meat flavour formation.** Reading / Inglaterra, 1994. 206p. (Tese de Doutorado - University of Reading, 1994).

(20) MADRUGA, M.S. Análise de inosina-5'-monofosfato (5'-imp) e seus derivados em diferentes músculos de bovinos, suinos e ovinos. **Ciênc. Tecnol. Aliment.**, in press, 1997.

(21) MADRUGA, M.S. & MOTTRAM, D.S. The effect of pH and inosine-5'-monophosphate (5'-IMP) on the formation of heterocyclic aroma volatiles in cooked meat. **Ciên. Tecnol. Alimentos**,v.15, p.284-287, 1995a.

(22) 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.**, v.68, p.305-310, 1995b

(23) MOTTRAM, D.S. Meat flavour: a review. In: PIGGOTT, J.R. & PATTERSON, A. **Understanding Natural Flavours**, Glasgow: Blackie,1994a, p. 140-163.

(24) MOTTRAM, D.S. Some aspects of the chemistry of meat flavour. In: SHAHIDI, F. **The Flavour of Meat and Meat Products**,. Glasgow: Blackie, 1994b, p.210-230.

(25) MOTTRAM, D.S. Meat. In: MAARSE, H. **Volatile Compounds in Foods and Beverages**, New York: Marcel Dekker, 1991, p.107-177.

(26) MOTTRAM, D. S.; WHITFIELD, F. B. Aroma volatiles from meat-like Maillard systems. In: McGORRIN, R.J.; PARLIMENT, T.H. & MORELLO, M.J.. **Microwave, Extruded and Maillard Generated Aromas**, Washington DC: American Chemical Society,1994, p 180-191.

(27) MOTTRAM, D. S.; MADRUGA, M.S.; WHITFIELD, F. B. Some Novel Meat-like Aroma Compounds from the Reactions of Alkanediones with Hydrogen Sulfide and Furanthiols. **J. Agric. Food Chem.**, v.43, p.189-193, 1995.

(28) MOTTRAM, D.S. & MADRUGA, M.S. Important sulfur-containing compounds in foods. In: MUSSINAN, C.J. & KEELAN, M. **Sulfur Compounds in Foods**, Washington: American Chemical Society, 1994a, p. 180-187.

(29) MOTTRAM, D.S. & MADRUGA, M.S. The role of inosine monophosphate as a precursor of meat aroma. In: MAARSE, H. & van der HEIJ, D.G. **Trends in Flavour Research**, Amsterdan: Elsevier Science, 1994b,p. 339-344.

(30) MOTTRAM, D.S. & EDWARDS, R.A. The role of triglycerides and phospholipids in the aroma of cooked beef. **J. Sci. Food Agric.**,v.34, p.517-522, 1983.

(31) PETERSON, R.J., IZZO, H.J., JUNGEMANN, E. & CHANG, S.S. Changes in volatile flavor compounds during the retorting of canned beef stew. **J.Food Sci.** v.40, p. 948-954, 1975.

(32) SHU, C.K., HAGEDOM, M.L., MOOKHERJEE, B.D. & HO, C.T. pH effect on the volatile components in the thermal degradation of cysteine. **J. Agric. Food Chem.**, v. 33, p. 442-446, 1985.

(33) van den OUWELAND, G.A.M. & PEER, H.G. Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3(2H)-furanone and its thio analog with hydrogen sulfide. **J. Agric. Food Chem.**, v.23, p.501-505, 1975.

(34) van GEMERT, L.J. & NETTENBREIJER, A.H. **Compilation of Odor Threshold Values in Air and Water.** The Netherlaands: TNO-CIVO Food Analysis Institute, 1977. 257p.

(35) WERKHOFF, P., BRUNING, J., EMBERGER, R., GUNTERT, M. & HOPP, R. Flavor chemistry of meat volatiles: New results on flavor components from beef, pork and chicken. In: HOPP, R. & MORI, K. **Recent developments in flavor and fragrance chemistry**, Weinheim: VHC, 1993, p.183-213.

(36) WERKHOFF, P., BRUNING, J., EMBERGER, R., GUNTERT, M.; KOPSEL, M.; KUHN, W.;SURBURG, H. Isolation and characterisation of volatile sulphur-containing meat flavour components in model systems. **J. Agric. Food Chem.** v.*38*, p.777-791, 1990.

(37) WHITFIELD, F. B.; MOTTRAM, D. S.; SHAW, K. J. The formation of some novel thiols and disulphides in model systems containing 4-hydroxy-5-methyl-3(2H)-furanone. In: SCHREIER, P. & WINTERHALTER, P. **Progress in Flavour Precursor Studies**, Carol Steram IL: Allured Publ. Co.,1993, p 395-400.

(38) ZHANG, Y.; HO, C. T. Formation of meatlike aroma compounds from thermal reaction inosine 5'-monophosphate with cysteine and glutathione. **J. Agric. Food Chem**, v.39, p.1145-8, 1991.