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Aroma compounds generated from thermal reaction of L-ascorbic acid with L-cysteine

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ABSTRACT

The reaction of L-ascorbic acid with L-cysteine in heated aqueous solution (141 ± 1 °C) at five different pH values (5.00, 6.00, 7.00, 8.00, or 9.00) for 2 h, resulted in the formation of a complex mixture of aroma volatiles. The volatile compounds generated were analysed by SPME–GC–MS. The results gave 43 aroma compounds. The reaction between L-ascorbic acid and L-cysteine led mainly to the formation of alicyclic sulphur compounds, thiophenes, thienothiophenes, thiophenones, thiazoles and pyrazines, most of which contain sulphur. Many of these volatiles had meaty flavour. The origin of many of the compounds was explained. The studies showed that thienothiophenes and thienones were formed mainly at acidic pH. In contrast, higher pH values could promote the production of thiophenes, thiazoles and pyrazines.

1. Introduction

The Maillard reaction is, together with lipid oxidation, without doubt the most important source of aroma compounds generated when food is cooked, baked, or roasted. The flavour industry makes use of the Maillard reaction to produce meat-like, cocoa-like, and other process flavours. L-Ascorbic acid (ASA) is a common ingredient of the human diet, occurring especially in fruit and vegetables, herbs, and to a lesser extent in meat (liver). In addition, ascorbic acid is frequently used as a food additive, as an antioxidant and as a flour improver in bakeries. As mentioned in our previous paper (Yu & Zhang, 2010), after reducing carbohydrates, ASA appears to be the most widely-studied carbonyl component in the processes of non-enzymatic browning, and a series of researches on the behaviour of ASA in the presence of amino acids *via* the Maillard reaction is reported in the literature.

However, there is lack of research findings on the formation of aroma compounds. As far as we know, data on the formation of aroma compounds produced by heating a model system containing ASA with L-cysteine (Cys) have not been found. Up to now, there is only one paper related to formation of aroma compounds in the model reactions of ASA with Cys (Adams & De Kimpe, 2009). Adams and De Kimpe reported formation of furan derivatives and thiophenes produced by heating a model reactions of ASA with Cys under dry-roasting conditions in the presence of K_2CO_3, but data were not shown. Sulphur-containing amino acids, such as Cys, are indispensable components for generating meat-like aromas through the Maillard reaction (Werkhoff et al., 1990). The present study was undertaken to study the effect of pH on the aroma compounds, especially meat-like aroma compounds, that are formed in the Maillard reaction between Cys and ASA.

2. Materials and methods

2.1. Reagents

ASA (analytical grade) was from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China), Cys was from Shanghai Yuanju Biological Technology Co., Ltd. (Shanghai, China). C_5–C_{22} *n*-alkanes were from Pure Chemical Analysis Co., Ltd. Na_2HPO_4, NaH_2PO_4 and NaOH were of analytical grade. Authentic samples for use as GC reference compounds were from J&K Chemical Ltd. (Beijing, China).

2.2. Model reaction of Cys with ASA

ASA (4.0 mmol) was dissolved in 40 ml of phosphate buffer (0.2 M), and the pH of the solution was adjusted to 5.00, 6.00, 7.00, 8.00, or 9.00 using NaOH. Cys (4.0 mmol) was added to

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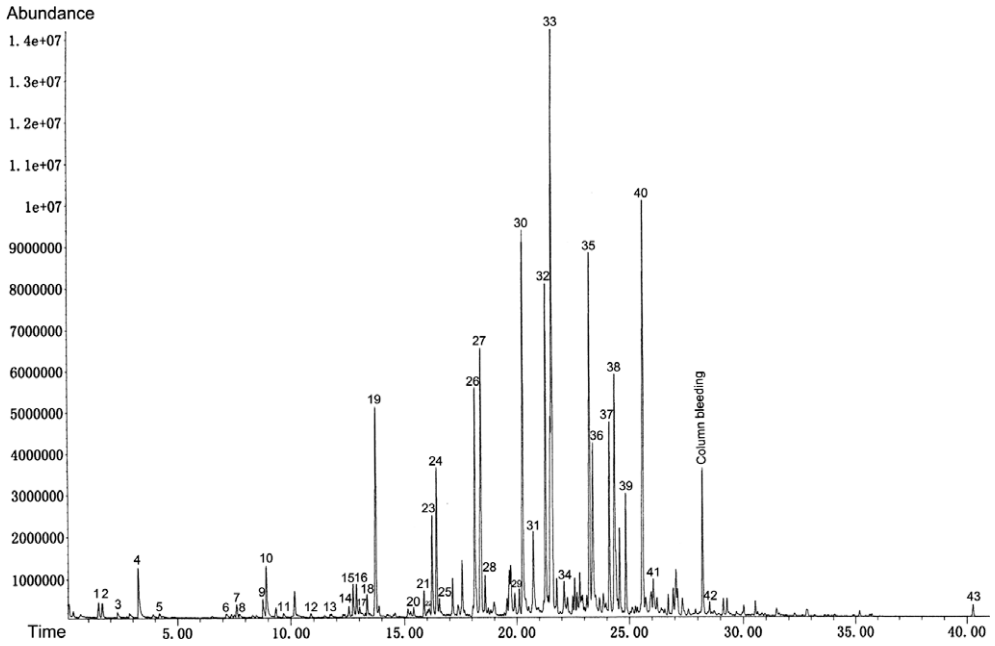

Fig. 1. Total ion chromatogram of aroma compounds produced by heating a model system containing L-ascorbic acid with L-cysteine at pH 7 and 141 ± 1 °C for 2 h using a CAR/PDMS fibre. Peak numbers correspond to Table 1.

the solution. The mixtures were then sealed in 48-ml Synthware® glass vials (Beijing Synthware Glass, Inc., Beijing, China) and heated while stirring at 141 ± 1 °C for 2 h in an oil bath. The reactions were immediately stopped by cooling under a stream of cold water.

2.3. Headspace-SPME–GC–MS

The assayed fibres were divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm thickness) and Carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm thickness; Supelco, Bellefonte, PA). Before the SPME fibre was inserted into the vial, the sample was equilibrated for 15 min at 40 °C. The extraction time was 50 min at 40 °C.

Analyses were performed using an Agilent 6890N gas chromatograph coupled to an Agilent 5975i mass selective detector (Agilent, Santa Clara, CA). Volatiles were separated using a DB-5 capillary column (30 m × 0.25 mm (i.d) × 0.25 μm). The SPME fibre was desorbed and maintained in the injection port at the temperature (250 °C) and for the time (4.0 min) suggested by the manufacturer. The injection port was in split mode and split ratio was 1:30. The temperature program was isothermal for 5 min at 40 °C, raised to 260 °C at a rate of 5 °C/min and then raised to 280 °C at a rate of 15 °C/min and held for 1 min. C_5–C_{22} *n*-alkanes were run under the same chromatographic conditions as the samples to calculate the linear retention indices (LRI) of detected compounds. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained using a mass selective detector (70 eV, multiplier voltage of 1753 V, collecting data at a rate of 1 scan/s over an *m*/*z* range of 30–400 amu). Compounds were identified by comparing their mass spectra with those contained in the NIST05 and Wiley275 libraries and by comparison of their LRI with those reported in the literature, as well as, whenever possible, GC co-injection with authentic samples available in our laboratories. Area counts of volatiles were provided by integrating at initial threshold 16.5, using Agilent Chemstation. Analysis of each tested condition was repeated twice and expressed as mean values.

3. Results and discussion

The reaction mixtures of the model system involving ASA with Cys at five different pHs possessed a different degrees of meat-like aromas. Total ion chromatogram of aroma compounds produced by heating a model system containing ASA with Cys at pH 7 and 141 ± 1 °C for 2 h using a CAR/PDMS fibre are shown in Fig. 1. The major headspace components of the model system involving ASA with Cys at five different pHs (5, 6, 7, 8, 9) are listed in Table 1. The 43 compounds presented were those which gave significant peaks by GC–TIC. They can be classified as ketones, thiazoles, pyrazines, thienones, thienothiophenes, thiophenes, alicyclic S-compounds, etc. The identifications were achieved by comparing their mass spectra with those contained in the NIST05 and Wiley275 libraries and comparing LRI of compounds peak Nos. 1–24; 26–30; 37,38, 42 and 43 with published data (NIST Chemistry Web Book, 2009); the identities of compounds peak Nos. 3, 6, 11, 13, 15, 19, 4, 9, 10, 12, 14, 30, 23, 24, 28 and 42 (Table 1) were confirmed by means of co-injection with authentic samples (Co-GC). The mass spectra of thieno[2,3-b]thiophene and thieno[3,2-b]thiophene were very similar; the identifications were achieved by their elution order on DB-5, according to literature (Mottram & Whitfield, 1995). When neither published LRI information nor authentic samples were available, the identification was established by comparing the fragmentation pattern of mass spectra with published data (NIST Chemistry Web Book, 2009). Table 2 listed the mass spectral data of these tentatively identified compounds formed from ASA and Cys. The effect of pH on the formation of aroma compounds followed similar patterns by using DVB/CAR/PDMS fibre and CAR/PDMS fibre.

In short, Table 1 showed that total amounts of thiazoles, pyrazines and thiophenes increased with increasing pH, and thienones and thienothiophenes decreased with increasing pH. The results indicated that high pH conditions favoured the formation of thiazoles, pyrazines and thiophenes. As mentioned above, furan derivatives and thiophenes (data not shown) were detected in a model reactions of ASA with Cys under dry-roasting conditions in the presence of K_2CO_3, but no pyrazines were detected (Adams & De

Table 1
Influence of the pH on the formation of aroma compounds from L-ascorbic acid and L-cysteine (GC-TIC peak areas × 10^6).

Peak No.[a]	Compound	LRI [b]	Identification	pH 5.0		pH 6.0		pH 7.0		pH 8.0		pH 9.0	
				D/C/P[c]	C/P[d]	D/C/P	C/P	D/C/P	C/P	D/C/P	C/P	D/C/P	C/P
1	3-Hexanone	<800	MS,LRI	ND[e]	11.1	ND	18.2	ND	13.1	ND	9.8	ND	8.3
2	2-Hexanone	<800	MS,LRI	ND	ND	ND	7.2	ND	13.3	ND	12.3	ND	11.6
7	2-Heptanone	888	MS,LRI	ND	2.7	ND	7.1	ND	10.9	ND	11.1	ND	10.1
	Total ketones			0	13.8	0	32.5	0	37.3	0	33.2	0	30.0
3	2-Methylthiazole	804	MS,LRI,Co-GC [f]	ND	2.8	ND	6.6	ND	5.8	ND	5.4	ND	5.2
5	5-Methylthiazole	845	MS,LRI	ND	2.7	ND	17.6	ND	3.9	ND	2.0	ND	1.8
6	2,4-Dimethylthiazole	880	MS,LRI,Co-GC	ND	ND	ND	2.2	ND	5.8	ND	8.5	ND	ND
8	2-Ethylthiazole	889	MS,LRI	ND	ND	ND	1.1	ND	3.6	ND	3.7	ND	4.2
11	4,5-Dimethylthiazole	926	MS,LRI,Co-GC	ND	ND	ND	1.0	ND	5.1	ND	5.2	ND	6.3
13	2-Ethyl-4-methylthiazole	967	MS,LRI,Co-GC	ND	ND	ND	ND	ND	1.9	ND	2.5	ND	3.3
15	2,4,5-Trimethylthiazole	988	MS,LRI,Co-GC	2.2	10.6	3.9	18.6	5.1	24.6	5.6	26.1	5.8	23.9
18	5-Ethyl-2-methylthiazole	1001	MS,LRI	2.6	17.9	2.4	11.0	2.4	17.3	2.7	22.7	4.1	20.2
19	2-Acetylthiazole	1011	MS,LRI,Co-GC	8.6	43.9	22.2	84.0	35.3	174.8	39.8	193.3	42.7	180.4
20	4-Ethyl-2,5-dimethylthiazole	1055	MS,LRI	2.6	6.4	2.8	6.1	2.9	5.9	2.8	5.6	2.3	5.2
21	5-Ethyl-2,4-dimethylthiazole	1067	MS,LRI	6.3	13.4	6.6	15.5	9.1	19.3	8.7	20.2	9.6	19.5
	Total thiazoles			22.3	97.7	37.9	163.7	54.8	268.0	59.6	295.2	64.5	270.0
4	Methylpyrazine	819	MS,LRI,Co-GC	ND	1.8	ND	16.7	5.8	51.2	4.9	65.6	12.4	74.1
9	2,6-Dimethylpyrazine	907	MS,LRI,Co-GC	ND	ND	ND	ND	0.4	15.7	2.8	25.4	3.3	27.7
10	Ethylpyrazine	910	MS,LRI,Co-GC	ND	ND	ND	ND	4.4	46.0	7.4	69.6	8.5	73.8
16	2-Ethyl-6-methylpyrazine	991	MS,LRI	ND	ND	ND	2.8	3.0	27.1	6.3	44.8	8.4	48.4
17	2-Ethyl-5-methylpyrazine	994	MS,LRI	ND	ND	ND	ND	ND	8.8	1.6	11.2	2.4	11.7
22	2,6-Diethylpyrazine	1072	MS,LRI	ND	ND	ND	ND	0.9	5.0	1.9	10.2	2.9	10.4
	Total pyrazines			0	1.8	0	19.5	14.5	153.8	24.9	226.8	37.9	246.1
12	Tetrahydrothiophen-3-one	950	MS,LRI,Co-GC	1.3	5.2	1.6	6.2	ND	3.5	ND	2.6	ND	3.9
14	2-Methyltetrahydrothiophen-3-one	985	MS,LRI,Co-GC	14.5	33.5	10.7	26.7	3.2	8.4	2.9	5.5	1.7	5.1
	Total thienones			15.8	38.7	12.3	32.9	3.2	11.9	2.9	8.1	1.7	9.0
29	Thieno[2,3-b]thiophene	1192	MS,LRI	18.5	39.5	15.9	29.5	9.2	24.9	7.7	20.9	7.6	13.9
30	Thieno[3,2-b]thiophene	1197	MS,LRI,Co-GC	192.5	420.1	131.3	270.3	130.9	303.7	128.8	259.2	132.5	231.1
36	2-Methylthieno[3,2-b]thiophene	1305	MS	152.6	222.4	130.0	162.5	126.7	171.1	111.8	137.0	112.4	103.9
41	2-Ethylthieno[2,3-b]thiophene	1402	MS	33.8	30.3	43.8	32.4	34.2	39.0	41.9	29.4	40.8	18.9
	Total thienothiophenes			397.4	712.3	321.0	494.7	301.0	538.7	290.2	446.5	293.3	367.8
23	3-Acetylthiophene	1077	MS,LRI,Co-GC	11.0	31.5	11.1	28.7	22.6	79.9	32.0	108.5	39.8	105.2
24	2-Acetylthiophene	1082	MS,LRI,Co-GC	3.1	7.2	3.8	13.8	28.8	111.2	48.6	169.4	59.8	168.7
25	2-Propyltetrahydrothiophene	1085	MS	ND	ND	ND	ND	5.6	13.2	9.4	31.5	13.6	24.1
28	2-Acetyl-3-methylthiophene	1146	MS,LRI,Co-GC	2.3	5.0	3.3	7.7	9.8	30.9	14.4	43.7	15.3	42.9
31	4-Hydroxybenzothiophene	1213	MS	14.0	17.3	28.6	32.5	64.6	91.5	51.7	68.1	46.4	46.5
34	3-Acetyl-2,5-dimethylthiophene	1261	MS	ND	ND	20.1	33.8	21.2	36.9	30.8	37.6	32.7	26.7
35	3-(Vinylthio)thiophene	1300	MS	157.8	223.8	159.8	191.2	200.6	288.8	210.5	280.6	214.1	263.3
	Total thiophenes			188.2	284.8	226.7	307.7	353.2	652.4	397.4	739.4	421.7	677.4
26	3,5-Dimethyl-1,2,4-trithiolane (*cis* or *trans*)	1132	MS,LRI	274.8	315.0	297.5	343.1	157.4	182.2	92.7	120.9	100.1	128.0
27	3,5-Dimethyl-1,2,4-trithiolane (*cis* or *trans*)	1140	MS,LRI	370.9	459.6	376.3	467.4	185.0	247.1	83.6	168.4	94.0	182.8
32	4,6-Dimethyl-1,2,3-trithiane (*cis* or *trans*)	1232	MS	40.7	40.8	130.0	135.7	275.6	254.7	223.2	240.1	246.5	238.9
33	4,6-Dimethyl-1,2,3-trithiane (*cis* or *trans*)	1242	MS	128.3	133.4	312.9	302.4	502.6	488.1	345.3	406.2	375.6	436.5
37	3,5-Diethyl-1,2,4-trithiolane (*cis* or *trans*)	1332	MS,LRI	53.8	46.1	94.4	74.1	168.4	141.0	181.9	142.7	190.8	123.5

Table 1 (*continued*)

Peak No.[a]	Compound	LRI[b]	Identification	pH 5.0		pH 6.0		pH 7.0		pH 8.0		pH 9.0	
				D/C/P[c]	C/P[d]	D/C/P	C/P	D/C/P	C/P	D/C/P	C/P	D/C/P	C/P
38	3,5-Diethyl-1,2,4-trithiolane (*cis* or *trans*)	1340	MS,LRI	73.1	60.5	136.4	104.7	270.9	237.5	205.1	155.7	239.8	200.1
40	1,2,5,6-Tetrathiocane ($C_4H_8S_4$)	1386	MS	377.0	359.9	442.6	350.7	395.3	352.5	214.7	173.3	195.6	138.4
43	Cyclic octaatomic sulphur (S_8)	>1800	MS,LRI	ND	7.1	2.0	7.0	6.4	8.6	8.8	8.4	7.0	7.5
	Total alicyclic S-compounds			1318.6	1422.4	1792.1	1785.1	1961.6	1911.7	1355.3	1415.7	1449.4	1455.7
39	4-Methyl-1,2-benzenedithiol	1358	MS	28.3	32.4	55.1	55.7	76.7	87.6	52.7	56.0	53.7	46.7
42	2,6-Di-*tert*-butyl-*p*-cresol (BHT)	1502	MS,LRI,Co-GC	13.9	12.1	14.5	8.5	16.0	9.6	19.2	7.1	17.3	3.9
	Total others			42.2	44.5	69.6	64.2	92.7	97.2	71.9	63.1	71.0	50.6

[a] Peak Nos. correspond to Fig. 1.
[b] LRI calculated for a DB-5 capillary column; mean values.
[c] DVB/CAR/PDMS fibre.
[d] CAR/PDMS fibre.
[e] Not detected.
[f] Co-injection with authentic sample.

Kimpe, 2009). This could be attributed to the different reaction conditions, for instance, with or without solvent, of different pHs, and of different reaction temperatures and reaction time.

3.1. Alicyclic S-compounds

A significant number of alicyclic S-compounds, especially *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolane, *cis*- and *trans*-4,6-dimethyl-1,2,3-trithiane, *cis*- and *trans*-3,5-diethyl-1,2,4-trithiolane, and 1,2,5,6-tetrathiocane, was identified in the model systems. Among detected alicyclic S-compounds, *cis*- and *trans*-3,5-diethyl-1,2,4-trithiolane increased with increasing pH, reaching a maximum at pH 7–8. The amounts of *cis*- and *trans*-4,6-dimethyl-1,2,3-trithiane increased with increasing pH, reached a maximum at pH 7; *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolane and 1,2,5,6-tetrathiocane reached a maximum at pH 6, then decreasing with increasing pH. Many alicyclic S-compounds have been found in Maillard model systems. The *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolanes are important meaty flavour components and have been characterised as meaty, boiled beef at low concentration, and onion-like and sulfurous at high concentration (Ohloff & Flament, 1978). They have been identified in various cooked foods (Mottram, 1994). They are the major volatile products in the reaction of glucose with cysteine (Zhang & Ho, 1991). These alicyclic S-compounds can be formed from the condensation of aldehydes, H_2S, and mercaptoacetaldehyde (Chen & Ho, 2002), all of which are thermal breakdown products of cysteine and ASA (Mottram & Whitfield, 1995; Vernin, Chakib, Rogacheva, Obretenov, & Párkányi, 1998). It has been suggested that 3,5-dimethyl-1,2,4-trithiolane can be formed by the reaction of two molecules of acetaldehyde with hydrogen sulphide (Takken, van der Linde, de Valois, van Dort, & Boelens, 1976). 3,5-Diethyl-1,2,4-trithiolane could also be formed in a similar reaction sequence by the reaction of propanal with hydrogen sulphide. The propanal can be derived from the decomposition of ASA (Vernin et al., 1998).

3.2. Thiophenes

Many thiophenes were detected in the systems. 3-(Vinylthio)thiophene, 3-acetylthiophene, 2-acetylthiophene, 2-propyltetrahydrothiophene, 2-acetyl-3-methylthiophene and 3-acetyl-2,5-dimethylthiophene increased with increasing pH. The results indicated that high pH conditions favoured the formation of these thiophenes. 2-Acetylthiophene, 3-acetylthiophene and 2-acetyl-3-methylthiophene have been identified in roasted coffee and thermal reaction of ribose and cysteine (Chen, Xing, Chin, & Ho, 2000; Vitzthum & Werkhoff, 1976). 3-Acetylthiophene and 2-acetylthiophene are described as being pungent and greensweet. 2-Acetyl-3-methylthiophene is described as imparting a honey-like flavour to syrup bases, and 3-acetyl-2,5-dimethylthiophene is recommended for improving the aroma of tobacco and perfumes (Yaylayan, 2000). The main routes for thiophene formation involve the reaction of furfural with hydrogen sulphide or the condensation of mercaptoacetaldehyde with α,β-unsaturated aldehydes (Madruga & Mottram, 1998).

3.3. Thienothiophenes

Four thienothiophenes were found in these studies. These compounds included thieno[2,3-b]thiophene, thieno[3,2-b]thiophene, 2-methylthieno[3,2-b]thiophene and 2-ethylthieno[2,3-b]thiophene. Thieno[2,3-b]thiophene and thieno[3,2-b]thiophene have been identified from Maillard reaction products (Chen & Ho, 2002) and have been synthesised. Thieno[2,3-b]thiophene and 2-methylthieno[3,2-b]thiophene have been identified in the headspace of different reactant ratios of ribose-cysteine mixtures

Table 2
Mass spectral data of some tentatively identified compounds from L-ascorbic acid and L-cysteine.

Peak No.[a]	Compound	LRI[b]	Major MS data, *m/z* (relative intensity)
1	3-Hexanone	<800	57 (100), 43 (96), 71 (77), 100 (58, M^+), 41 (27), 39 (14), 42 (9), 72 (8), 44 (7); MW = 100
2	2-Hexanone	<800	43 (100), 58 (66), 32 (57), 57 (21), 100 (18, M^+), 41 (17), 85 (14), 39 (9), 71 (8), 42 (7); MW = 100
36	2-Methylthieno[3,2-b]thiophene	1305	153 (100), 154 (65, M^+), 155 (15), 69 (9), 156 (6), 77 (5), 109 (5), 45 (4), 121 (4), 32 (3); MW = 154
41	2-Ethylthieno[2,3-b]thiophene	1402	153 (100), 168 (43, M^+), 32 (15), 154 (10), 155 (10), 69 (7), 167 (6), 169 (6), 109 (5), 170 (5); MW = 168
25	2-Propyltetrahydrothiophene	1085	87 (100), 130 (29, M^+), 45 (17), 43 (15), 85 (13), 53 (9), 59 (9), 39 (5); MW = 130
31	4-Hydroxybenzothiophene	1213	150 (100, M^+), 121 (74), 122 (22), 96 (10), 78 (10), 77 (10), 151 (10), 63 (6), 69 (6); MW = 150
34	3-Acetyl-2,5-dimethylthiophene	1261	139 (100), 154 (46, M^+), 141 (13), 140 (10), 67 (8), 97 (6), 45 (5), 69 (5); MW = 154
35	3-(Vinylthio)thiophene	1300	142 (100, M^+), 141(81), 97 (43), 45 (13), 69 (11), 144 (9), 140 (6), 71 (4), 70 (4); MW = 142
32,33	4,6-Dimethyl-1,2,3-trithiane (*cis* or *trans*)	1232	166 (100, M^+), 102 (33), 60 (30), 69 (29), 59 (28), 101 (26), 45 (25), 64 (21), 92 (21), 41 (20); MW = 166
40	1,2,5,6-Tetrathioctane ($C_4H_8S_4$)	1386	59 (100), 184 (79, M^+), 60 (50), 124 (48), 64 (19), 45 (18), 119 (17), 186 (14), 58 (12), 61 (10); MW = 184
43	Cyclic octaatomic sulphur (S_8)	>1800	32 (100), 64 (68), 256 (62, M^+), 160 (40), 128 (39), 192 (26), 258 (23), 96 (17), 162 (9); MW = 256
39	4-Methyl-1,2-benzenedithiol	1358	156 (100, M^+), 155 (76), 123 (16), 157 (16), 121 (13), 141 (12), 111 (11), 45 (10), 158 (9); MW = 156

[a] Peak nos. correspond to Fig. 1.
[b] LRI calculated for a DB-5 capillary column; mean values.

treated with and without supercritical-CO_2 (Xu, Liu, Zhao, & Gao, 2008). High concentrations of several derivatives of thienothiophene were determined in these reaction mixtures, especially thieno[3,2-b]thiophene and 2-methylthieno[3,2-b]thiophene. The amounts of thienothiophenes decreased with increasing pH. Cys was thermally stable when heated alone under dry conditions; however, heating Cys on its own in the presence of a small amount of water led to the formation of thienothiophenes. The quantities were low, since the breakdown of amino acids in the Maillard reaction occurs more readily than thermal degradation in the absence of a sugar (Mottram & Whitfield, 1995). In this model system, a significant number of thienothiophenes, especially thieno[3,2-b]thiophene and 2-methylthieno[3,2-b]thiophene was identified. The results indicated that ASA plays a significant role and can promote the formation of thienothiophenes in this model system.

3.4. Thiophenones

Two thiophenones were detected in the model system involving ASA with Cys at five different pHs, i.e. tetrahydrothiophen-3-one and 2-methyltetrahydrothiophen-3-one. Tetrahydrothiophen-3-one is a well-known food volatile. Its formation from the amino acids cystine and cysteine by condensation of two molecules of mercaptoacetaldehyde (a main Strecker degradation product) is much easier to explain. The probable precursor of tetrahydrothiophene-3-one is 1,4-dimercapto-2-butanone (Güntert et al., 1990). Tetrahydrothiophen-3-one has been identified in pressure-cooked beef and yeast extract (Güntert et al., 1990). 2-Methyltetrahydrothiophen-3-one is another well-known flavour volatile. Its identification as a thermal degradation product of thiamin has been described previously (Hartman, Carlin, Scheide, & Ho, 1984). A possible pathway for the formation of 2-methyltetrahydrothiophen-3-one involves aldol condensation between acetaldehyde, derived from cysteine or ASA, and pyruvaldehyde, formed from ASA (Vernin et al., 1998). The aldol condensation product reacts with hydrogen sulphide to produce 2-methyltetrahydrothiophen-3-one.

3.5. Thiazoles

Eleven thiazoles were identified in the model reaction systems. The thiazoles identified in this study indicated that pH could influence their formation. The formation of thiazoles was favoured under basic conditions more than in an acidic environment, whereas 2-acetylthiazole was more prominent at pH 7–9. Thiazole compounds have been found in various food systems, and they contribute to a wide variety of characteristic aromas to foods. Monosubstituted alkylthiazoles, such as 2-methylthiazole, 5-methylthiazole and 2-ethylthiazole, possess green, vegetable aromas, whereas S-compounds such as 4,5-dimethylthiazole, 5-ethyl-2,4-dimethylthiazole, and 2,4,5-trimethylthiazole have been described as nutty, roasted, and meaty (Maga, 1975). Like pyrazines, their formation also requires heating at elevated temperatures. Therefore, they are often detected in fried, roasted, or grilled foods, such as cooked meats, coffee, roasted peanuts, and potato chips. For instance, 4,5-dimethylthiazole, trimethylthiazole, 2-acetylthiazole, and 5-ethyl-2,4-dimethylthiazole have been found in grilled meats (Melton, 1998). In general, thiazoles are mainly formed by non-enzymatic browning reactions between reducing sugars and amino acids in the presence of H_2S. Therefore, thiazoles can be identified in nearly all cooked or roasted food aromas. With the exception of 5-methylthiazole, 4,5-dimethylthiazole, 2-ethyl-4-methylthiazole and 2-acetylthiazole, all other thiazoles formed in the reaction of ASA with Cys have a methyl substituent in the 2-position. Accordingly, it is likely that one of the precursors to all of these compounds is acetaldehyde, derived from the decomposition of ASA (Vernin et al., 1998). Other compounds likely to be involved in the formation of such 2-methylthiazoles would be the ASA decomposition products pyruvaldehyde (for 2,4-dimethylthiazole) and 2,3-butanedione (for 2,4,5-trimethylthiazole) (Vernin et al., 1998). The key steps in the reaction sequences to these compounds are the reaction of ammonia with acetaldehyde to form 1-aminoethanol and the condensation of this compound with appropriate α-dicarbonyl compounds. The thiazoles could be formed from these condensation products by reaction with H_2S followed by cyclisation and dehydration (Vernin & Párkányi, 1982).

3.6. Pyrazines

Six pyrazines were identified in the model reaction systems. The quantities of pyrazines increased significantly as the pH increased. Some pyrazines were not identified at pH 5–6 except for methylpyrazine and 2-ethyl-6-methylpyrazine (Table 1). The results indicated that high pH conditions favoured the formation of pyrazines. When the pH of the reaction solution was higher than 6, the quantities of pyrazines increased obviously. The thermal degradation of ASA can produce many carbonyl compounds (Vernin et al., 1998). The base catalysis was probably due both to the increased reactivity of the amino group of the amino acid toward the carbonyl and to the increased rearrangement and fragmentation of ASA (Koehler & Odell, 1970). Pyrazines may contribute to toasted, roasted, nutty, and burnt notes. There are several precursors or pathways for pyrazine compounds. The α-amino carbonyls, which can be formed from the reactions between dicarbonyl compounds and amino acids during Strecker degradation, are generally

considered to be the precursors of pyrazines. The dicarbonyl compounds such as ethylglyoxal, butanedione, glyoxal, pyruvaldehyde can be produced by thermal degradation of ASA (Vernin et al., 1998). Cys can release ammonia when heated in an aqueous solution (Sohn & Ho, 1995). During Maillard reactions and thermal degradation of ASA, some active intermediates such as 2-hydroxypropanal, hydroxyacetaldehyde, hydroxyacetone, acetoin can be produced (Vernin et al., 1998). These intermediates react with ammonia to generate α-aminoketones and then form pyrazines. These α-aminocarbonyls may react with each other to generate pyrazines during thermal processing (Wang & Odell, 1973).

3.7. Compounds that probably contribute to the meaty flavour

Above, we discussed that *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolanes, 4,5-dimethylthiazole, 5-ethyl-2,4-dimethylthiazole and 2,4,5-trimethylthiazole had meat-like flavour. In addition, among the volatile compounds, 2-acetylthiazole, 2,4-dimethylthiazole, methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-acetylthiophene have been reported to be important volatile compounds, which could contribute to meaty flavour (Yu, Wu, & Ho, 1994). Tetrahydrothiophen-3-one and 2-methyltetrahydrothiophen-3-one are also meat-like aroma compounds. No single one of these compounds may be considered to be the character impact compound for meaty flavour generated in model systems; however, the combination of these compounds and probably other compounds identified may lead to meaty flavour sensation.

In conclusion, the results have shown that the reaction between ASA and Cys leads mainly to the formation of alicyclic S-compounds, thiophenes, thienothiophenes, thiophenones, thiazoles and pyrazines, most of which contain sulphur. Many of these volatiles had meaty flavour. The influence of pH on the subsequent thermal flavour formation is absolutely significant. Thienothiophenes and thienones were formed mainly at acidic pH. In contrast, higher pH values could promote the production of thiophenes, thiazoles and pyrazines.

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