Unlocking the Potential of Cysteine-Xylose Maillard Reaction Intermediates as Natural Flavor Precursor: A Comprehensive Study on Flavor Regulation, Storage Stability, and Antioxidant Activity

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Supplementary data

Below are the supplementary data for the derivative fragmentation in the LC-MS/MS spectrum and ^{13}C NMR data of TTCA; The water activity corresponding to each saturated salt solution at 25°C; Calibration curves of volatile compounds; Volatile flavor compounds (μg/L) identified in the TTCA model reactions under elevated temperatures of 100, 120 and 140 ℃ at an initial pH value of 7.0; Volatile flavor compounds (μg/L) identified in the TTCA model reactions under different pH values of 5.5, 7 and 8 at the temperature of 120 ℃; Retention rate of TTCA/ARP under different conditions; The high performance liquid chromatogram of TTCA/ARP compounds and the total ions chromatogram (a)/LC-MS/MS spectra (b) of purified TTCA; The release of Cys during the degradation of TTCA; The depletion of Cys compound at an initial pH of 7 under different temperatures (100, 120 and 140 ℃).

Table S1. Derivative fragmentation in the LC-MS/MS spectrum and ^{13}C NMR data of TTCA

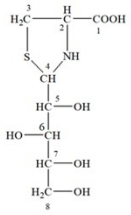
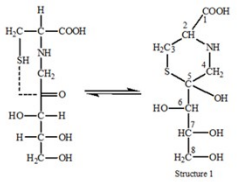
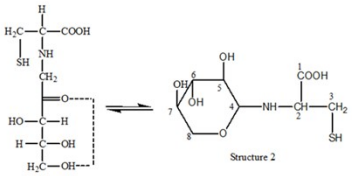
Compounds	Structures	^{13}C NMR/ppm	Ions in MS/MS spectra of $[M+H]^+$ (m/z)
TTCA		174.40 (s) (C1), 75.07 (d) (C6), 74.42 (d) (C5), 72.92 (d) (C7), 69.77 (d) (C2), 67.48 (d) (C4), 64.83 (t) (C8), 35.60(t) (C3)	254 $[M+H]^+$; 236 $[M+H-H_2O]^+$; 218 $[M+H-2H_2O]^+$; 200 $[M+H-3H_2O]^+$; 188 $[M+H-2H_2O-CH_2O]^+$
Cys-Amadori	 Structure 1	175.00 (s) (C_1), 82.79 (s) (C_5), 76.38 (d) (C_6), 73.20(d) (C_7), 66.62 (t) (C_3), 58.35 (t) (C_8), 55.51 (t) (C_4), 28.04 (t) (C_3)	254 $[M+H]^+$; 236 $[M+H-H_2O]^+$; 218 $[M+H-2H_2O]^+$; 200 $[M+H-3H_2O]^+$; 188 $[M+H-2H_2O-CH_2O]^+$
	 Structure 2	174.60.00 (s) (C_1), 82.17 (d) (C_4), 78.01 (d) (C_6), 72.93(d) (C_5), 66.52 (d) (C_7), 62.78 (t) (C_8), 51.40 (d) (C_2), 26.83 (t) (C_3)	

Table S2. Water activity corresponding to each saturated salt solution at 25°C.

saline	a_w
	25°C
lithium chloride	0.113 ± 0.003
potassium carbonate	0.432 ± 0.004
dicalcium phosphate	0.843 ± 0.003

Table S3. The calibration curves of volatile flavor compounds

Volatile flavor compounds	Calibration curves	R^2
Thiophene	$y = 0.0223\ x + 0.0011$	0.9991
2-Methylthiophene	$y = 0.0472\ x + 0.0009$	0.9994
Thiazole	$y = 0.0601\ x - 0.0012$	0.9982
2-Methylthiazole	$y = 0.0084\ x - 0.0521$	0.9923
3-Mercapto-2-butanone	$y = 0.1216\ x - 0.0007$	0.9999
2,5-Dimethyl-thiazole	$y = 0.0637\ x + 0.0012$	0.9979
2-Methyl-3-furanthiol	$y = 0.0039\ x + 0.0011$	0.9989
2-Ethyl-thiazole	$y = 0.0629x + 0.0041$	0.9994
3-Mercapto-2-pentanone	$y = 0.0379\ x + 0.0131$	0.9997
2,4,5-Trimethylthiazole	$y = 0.0571\ x - 0.0007$	0.9994
4,5-Dimethyl-thiazole	$y = 0.0297\ x - 0.0042$	0.9969
2-Furfurylthiol	$y = 0.0287\ x - 0.0003$	0.9992
5-Ethyl-2,4-dimethyl-thiazole	$y = 0.0184\ x + 0.0008$	0.9981
2-Ethyl-4-methyl-thiazole	$y = 0.0430\ x + 0.0024$	0.9923
3-Thiophenethiol	$y = 0.0285\ x + 0.0033$	0.9954
2-Acetylthiazole	$y = 0.0364\ x - 0.0014$	0.9967
2-Thiophenecarboxaldehyde	$y = 0.0446\ x + 0.0009$	0.9998
5-Methyl-2-thiophenecarboxaldehyde	$y = 0.0296\ x - 0.0007$	0.9999
2-Thiophenemethanethiol	$y = 0.0517\ x + 0.0012$	0.9954
1-(3-Thienyl)-ethanone	$y = 0.0728\ x - 0.00012$	0.9981
2,5-Thiophenedicarboxaldehyde	$y = 0.0423\ x - 0.0031$	0.9997
Methylpyrazine	$y = 0.0398\ x + 0.0012$	0.9949
Pyrazine	$y = 0.0294\ x + 0.0012$	0.9989
3-Methyl-pyridine	$y = 0.0563\ x + 0.0032$	0.9998
2,5-Dimethyl-pyrazine	$y = 0.0319\ x + 0.0013$	0.9978
Furan	$y = 0.0253\ x - 0.0012$	0.9968
2-Methyl-furan	$y = 0.0336\ x + 0.0021$	0.9997
Furfural	$y = 0.0347\ x - 0.0012$	0.9923
1-(2-Furanyl)-ethanone	$y = 0.0629\ x + 0.0024$	0.9948
2(5H)-Furanone	$y = 0.0598\ x - 0.0011$	0.9993
4-Hydroxy-5-methyl-3(2H)-furanone	$y = 0.0098\ x + 0.0002$	0.9971

Table S4. Volatile flavor compounds (μg/L) identified in the TTCA model reactions under elevated temperatures of 100, 120 and 140 ℃ at an initial pH value of 7.0.

Compounds	RI[a]	RI[b]	Concentration (μg/L)			ID[c]
			100℃	120℃	140℃	
Sulfur-containing compounds						
Thiophene	1016	1022	0.000	0.000	0.118±0.072	RI, MS
2-Methylthiophene	1135	1095	0.000	0.000	0.892±0.115	RI, MS
Thiazole	1240	1262	0.896±0.053c	1.796±0.12b	2.038±0.139a	RI, MS
2-Methylthiazole	1272	1278	0.142±0.008bc	0.194±0.014b	0.254±0.109a	RI, MS
3-Mercapto-2-butanone	1273	1283	1.303±0.153bc	1.798±0.127b	2.398±0.141a	RI, MS
2,5-Dimethyl-thiazole	1301	1326	0.079±0.013c	2.203±0.142b	2.553±0.096a	RI, MS
2-Methyl-3-furanthiol	1302	1305	1.237±0.142bc	1.388±0.256b	5.907±0.085a	RI, MS
2-Ethyl-thiazole	1319	1304	0.245±0.035b	0.273±0.006ab	0.289±0.025a	RI, MS
3-Mercapto-2-pentanone	1352	1343	0.973±0.099c	1.298±0.253b	1.736±0.234a	RI, MS
2,4,5-Trimethylthiazole	1373	1390	0.419±0.052c	3.199±0.075b	3.843±0.188a	RI, MS
2-Furfurylthiol	1426	1402	3.734±0.085c	4.107±0.137b	11.439±0.265a	RI, MS
5-Ethyl-2,4-dimethyl-thiazole	1437	-	0.000	0.587±0.078b	0.889±0.085a	RI, MS
2-Ethyl-4-methyl-thiazole	1449	1410	0.117±0.005c	1.278±0.117b	2.112±0.370a	RI, MS
3-Thiophenethiol	1564	1530	0.000	0.178±0.027b	0.268±0.062a	RI, MS
2-Acetylthiazole	1646	1643	3.079±0.215c	8.795±0.094b	9.858±1.215a	RI, MS
2-Thiophenecarboxaldehyde	1674	1679	0.09±0.013c	2.196±0.25b	2.28±0.144a	RI, MS
5-Methyl-2-thiophenecarboxaldehyde	1701	1785	0.936±0.06c	3.649±0.099b	5.706±0.644a	RI, MS
2-Thiophenemethanethiol	1702	1713	0.119±0.074c	0.196±0.015b	0.332±0.21a	RI, MS
2-Methyl-3-[(2-methyl-3-thienyl)dithio)furan	1732	-	0.021±0.004c	0.098±0.033b	0.137±0.036a	RI, MS
1,2,3-Trithiolane	1794	-	0.208±0.029c	0.378±0.071b	0.497±0.185a	RI, MS
3,3'-Dithiobisthiophene	1845	-	0.000	0.000	0.000	RI, MS
Thieno[3,2-b]thiophene	1868	1843	0.000	1.302±0.079b	3.369±0.454a	RI, MS
2,5-Thiophenedicarboxaldehyde	1911	-	0.346±0.039c	0.724±0.139b	1.283±0.122a	RI, MS
2-Methylthieno[2,3-b] thiophene	1947	-	0.000	0.19±0.03b	2.123±0.221a	RI, MS
Bis(2-furfuryl)sulfide	2419	-	0.034±0.007bc	0.042±0.004b	0.078±0.018a	RI, MS
Subtotal			**13.978±0.568c**	**35.866±1.382b**	**60.400±1.620a**	
Nitrogen-containing heterocycles						
Methylpyrazine	1214	1263	0.000	0.000	0.000	RI, MS
Pyrazine	1216	1209	0.000	0.000	0.000	RI, MS
3-Methyl-pyridine	1307	1346	0.000	0.000	0.000	RI, MS
2,5-Dimethyl-pyrazine	1320	1328	0.000	0.000	0.000	RI, MS
Subtotal			**0.000**	**0.000**	**0.000**	
Oxygen-containing heterocycles						
Furan	797	798	0.000	0.138±0.017a	1.108±0.092b	RI, MS
2-Methyl-furan	851	829	0.000	0.019±0.007b	0.133±0.088a	RI, MS
Furfural	1457	1460	3.381±0.089c	5.793±0.422b	11.039±0.302a	RI, MS
1-(2-Furanyl)-ethanone	1497	1501	0.000	0.136±0.017b	0.379±0.087a	RI, MS

2(5H)-Furanone	1748	1767	0.000	0.072±0.012b	0.098±0.015a	RI, MS
Subtotal			**3.381±0.089c**	**6.158±0.425b**	**12.757±0.469a**	RI, MS
Total			**17.359±0.527c**	**42.024±1.737b**	**73.157±1.557a**	RI, MS

Notes: Results were presented as means ± standard deviation, data within a row with different letters are significantly different ($p < 0.05$) using Duncan's multiple comparison test ($n = 3$).

a: Linear retention indices calculated using a series of n-alkanes on the DB-W AX column (30 m × 0.25 mm× 0.25 μm).

b: Linear retention indices searched from http:// www.flavornet.org and http://webbook.nist.gov/chemistry/.

c: ID: Identification methods.

d: "-", not detected.

Table S5. Volatile flavor compounds (μg/L) identified in the TTCA model reactions under different pH values of 5.5, 7 and 8 at the temperature of 120 ℃.

Compounds	RI[a]	Ri[b]	Concentration (μg/L)			ID[c]
			pH 5.5	pH 7	pH8	
Sulfur-containing compounds						
Thiols/Sulfides						
3-Mercapto-2-butanone	1273	1283	1.796±0.152a	1.303±0.006b	0.973±0.077c	RI, MS
2-Methyl-3-furanthiol	1302	1305	1.936±0.065a	1.237±0.061b	1.103±0.008c	RI, MS
3-Mercapto-2-pentanone	1352	1343	1.793±0.036a	0.973±0.025c	1.332±0.176b	RI, MS
2-Furfurylthiol	1426	1402	5.639±0.095a	3.734±0.074b	3.012±0.097c	RI, MS
3-Thiophenethiol	1564	1530	0.000	0.000	0.079±0.004a	RI, MS
2-Methyl-3-[(2-methyl-3-thienyl)dithio)furan	1732	-	0.179±0.014a	0.021±0.004b	0.000	MS
2-Thiophenemethanethiol	1947	-	0.211±0.007a	0.119±0.016b	0.034±0.004c	MS
Bis(2-furfuryl)sulfide	2419	-	0.057±0.007a	0.034±0.012b	0.000	MS
Subtotal			**11.611±0.123a**	**7.421±0.146b**	**6.533±0.143c**	
Thiophenes						
Thiophene	1016	1022	0.000	0.000	0.179±0.008a	RI, MS
2,3-Dihydro-5-methyl-thiophene	1133	1156	0.000	0.000	0.000	RI, MS
2-Methylthiophene	1135	1095	0.028±0.004a	0.000	0.000	RI, MS
2,5-Dimethyl-thiophene	1144	1190	0.000	0.000	0.193±0.014a	RI, MS
Dihydro-2-methyl-3(2H)-thiophenone	1497	1506	0.000	0.000	0.079±0.007a	RI, MS
Dihydro-3-(2H)-thiophenone	1542	1563	0.000	0.000	0.087±0.023a	RI, MS
2-Thiophenecarboxaldehyde	1674	1679	0.221±0.005a	0.09±0.007b	0.012±0.004c	RI, MS
Thieno[3,2-b]thiophene	1868	1843	0.000	0.000	0.196±0.011a	RI, MS
5-Methyl-2-thiophenecarboxaldehyde	1701	1785	0.837±0.01b	0.936±0.043a	0.423±0.016c	RI, MS
2-Acetyl-3-methylthiophene	1757	-	0.000	0.000	0.000	MS
3,3'-Dithiobisthiophene	1845	-	0.000	0.000	0.000	MS
2,5-Thiophenedicarboxaldehyde	1911	-	0.336±0.023a	0.346±0.027a	0.329±0.011a	MS
2-Methylthieno[2,3-b] thiophene	1947	-	0.000	0.000	0.189±0.02a	RI,
2,3'-Bithiophene	2194	-	0.000	0.000	0.023±0.007a	MS
Subtotal			**1.422±0.039b**	**1.372±0.068b**	**1.71±0.025a**	
Thiazoles						
Thiazole	1240	1262	0.334±0.012c	0.896±0.012b	2.137±0.054a	RI, MS
2-Methylthiazole	1272	1278	0.023±0.004b	0.142±0.007b	1.779±0.113a	RI, MS
2,5-Dimethyl-thiazole	1301	1326	0.000	0.079±0.015b	0.127±0.008a	RI, MS
2,4,5-Trimethylthiazole	1373	1390	0.112±0.067c	0.419±0.074b	1.192±0.021a	RI, MS
2-Ethyl-thiazole	1319	1304	0.000	0.245±0.012b	0.283±0.018a	RI, MS
2-Ethyl-4-methyl-thiazole	1449	1410	0.000	0.117±0.01b	0.217±0.007a	RI, MS
2-Acetylthiazole	1646	1643	0.238±0.008c	3.079±0.075b	5.179±0.085a	RI, MS
Subtotal			**0.707±0.07c**	**4.977±0.175b**	**10.914±0.226a**	
Other sulfur-containing compounds						

1,2,3-Trithiolane	1794	-	1.128±0.01c	0.208±0.009b	0.000	MS
Nitrogen-containing heterocycles						
Pyrazine	1210	1209	0.000	0.000	0.129±0.007a	RI, MS
Methylpyrazine	1214	1263	0.000	0.000	0.271±0.016a	RI, MS
3-Methyl-pyridine	1307	1346	0.000	0.000	0.037±0.013a	RI, MS
2,5-Dimethyl-pyrazine	1320	1328	0.000	0.000	0.438±0.033a	RI, MS
Pyrrole	1497	1507	0.000	0.000	0.021±0.002a	RI, MS
Subtotal			0.000	0.000	0.896±0.032a	
Oxygen-containing heterocycles						
Furan	797	798	0.796±0.115a	0.000	0.000	RI, MS
2-Melthy-furan	851	829	0.273±0.045a	0.000	0.000	RI, MS
Furfural	1457	1460	4.238±0.32a	3.381±0.34b	1.128±0.147c	RI, MS
Benzofuran	1493	-	0.000	0.000	0.000	MS
4-Hydroxy-5-methyl-3(2H)-furanone	2108	2124	0.000	0.000	0.000	RI, MS
Subtotal			5.307±0.459a	3.381±0.34b	1.128±0.147c	
Total content of sulfur-containing compounds			**14.868±0.096b**	**13.978±0.081c**	**19.157±0.353a**	
Total			**20.175±0.504a**	**17.359±0.421b**	**21.181±0.334a**	

Notes: Results were presented as means ± standard deviation, data within a row with different letters are significantly different ($p < 0.05$) using Duncan's multiple comparison test ($n = 3$).

a: Linear retention indices calculated using a series of n-alkanes on the DB-W AX column (30 m × 0.25 mm× 0.25 μm).

b: Linear retention indices searched from http:// www.flavornet.org and http://webbook.nist.gov/chemistry/.

c: ID: Identification methods.

d: "-", not detected.

Table S6. Retention rates of TTCA/ARP under different conditions

Conditions		Retention rate of TTCA (%)	Retention rate of ARP (%)
Temperature	4℃	99.12	95.52
	25℃	95.53	94.18
	40℃	92.94	87.83
pH	pH 5.5	95.51	94.23
	pH 7.0	96.7	90.52
	pH 9.0	88.81	78.75
a_w	0.113	86.49	78.48
	0.432	72.47	57.27
	0.843	64.23	39.39

Figure Captions

Figure S1. The high performance liquid chromatogram of TTCA/ARP compounds.

Figure S2. The total ions chromatogram (a)/LC-MS/MS spectra (b) of purified TTCA

Figure S3. The release of Cys during the degradation of TTCA.

Figure S4. Depletion of Cys compound at an initial pH of 7 under different temperatures (100, 120 and 140 ℃).

Fig. S1

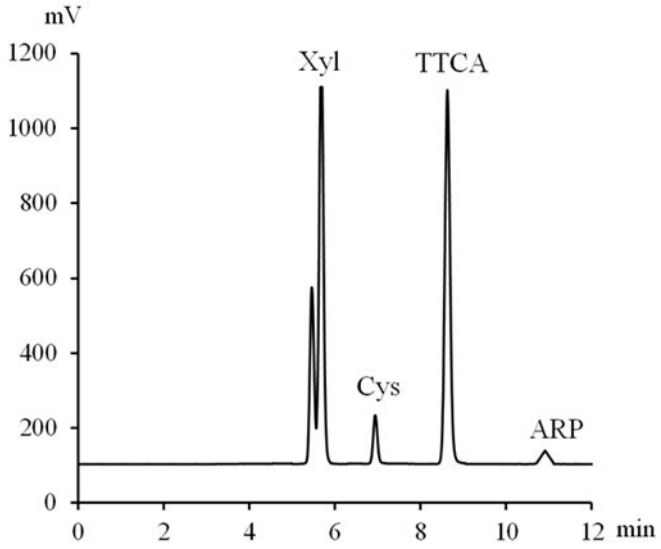
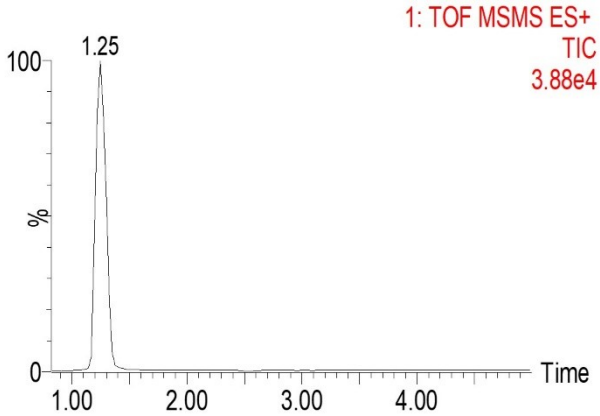


Fig. S2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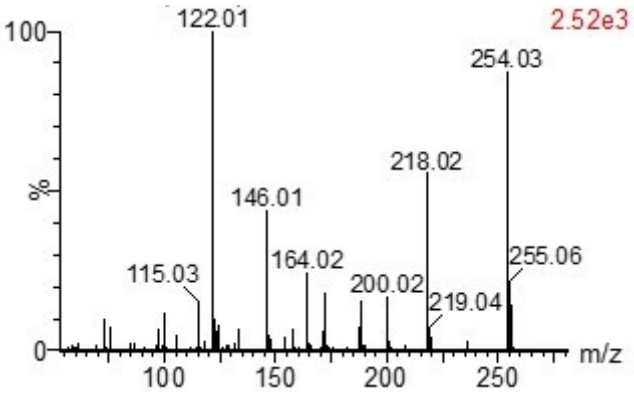
4.00
Time

(a)


2.52e3
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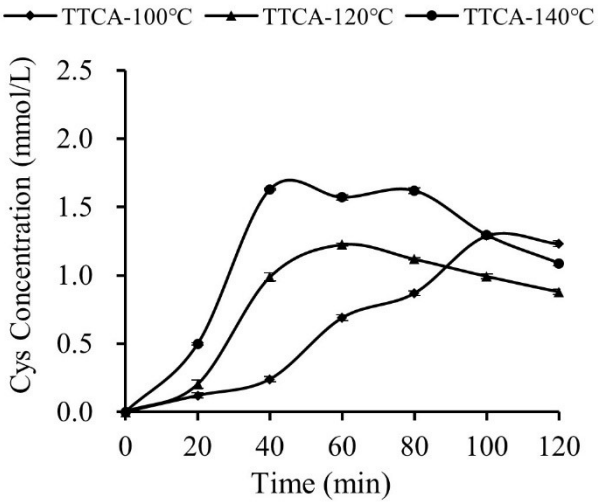
%
0
100
150
200
250
m/z

(b)

Fig. S3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TTCA-120℃
TTCA-140℃
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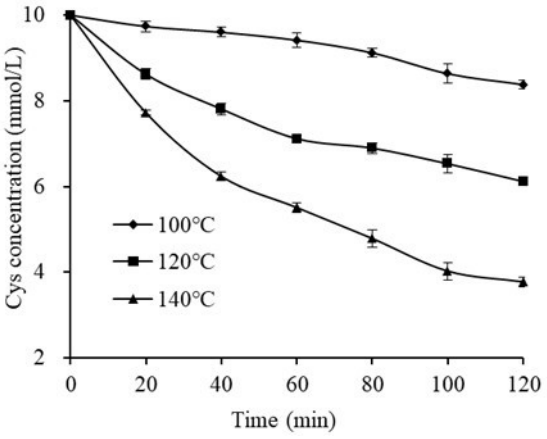
1.5
1.0
0.5
0.0
0
20
40
60
80
100
120
Time (min)

Fig. S4


Cys concentration (mmol/L)
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8
6
4
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0
20
40
60
80
100
120
Time (min)
100°C
120°C
140°C