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Impact of High Temperature on the Maillard Reaction between Ribose and Cysteine in Supercritical Carbon Dioxide

Honggao Xu, Wenhao He, Xuan Liu, and Yanxiang Gao*

College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China

Abstract An aqueous ribose-cysteine model system (initial pH 5.6) was conventionally heated to the same browning at varying temperatures (120-180°C), supercritical carbon dioxide (SC-CO_2, 20 MPa) was also applied on the same matrices for same periods at each temperature and about 20% reduction of the absorbance at 420 nm was observed as compared with sole thermal treatment. The headspace volatiles from Maillard reaction mixtures were analyzed by solid-phase microextraction (SPME) in combination with gas chromatography and mass spectrometry (GC-MS), and predominated with sulfur containing compounds, such as thienothiophenes, polysulfur alicyclics, thiols, and disulfides. Reaction temperature exhibited complex effects on volatiles formation and those effects became further complicated by the SC-CO_2 treatment. The formation of noncarbonyl polysulfur heterocyclic compounds and thienothiophenes was generally favored at high temperatures. Most volatiles were inhibited in SC-CO_2 as compared with thermal treatment alone, however, the well-known meaty aromatic compounds, such as thiols and disulfides, were obviously enhanced.

Keywords: high temperature, supercritical carbon dioxide (SC-CO_2), volatile compound, ribose, cysteine

Introduction

Ribose and cysteine are the most important precursors in manufacturing meat process flavorings (1,2). Aromatic components, generated from heated ribose-cysteine model system with or without other ingredients at different temperatures, were dominated with sulfur containing volatiles. Mottram and Whitfield (3,4) reported on heating ribose-cysteine model system at 185°C, either dry or low moisture, and the major volatiles were polysulfur heterocyclic compounds and thienothiophenes. When the same model system was heated at 140°C in different buffer solutions, the catalytic effect of buffers on keto-enol tautomerisms to form 4-hydroxy-5-methyl-3(2H)-furanone via 2,3-enolization was greater than that of pH (5). 4-Hydroxy-5-methyl-3(2H)-furanone was not an important intermediate for the formation of 2-methyl-3-furanthiol when the ribose-cysteine system reacted at 95°C (2,6), and 1,4-dideoxypentosone was proposed as a key intermediate. However, the information pertaining to the thermal effect on the volatile components in the same browning reaction mixtures, treated at different temperatures, was very limited.

Supercritical carbon dioxide (SC-CO_2), a benign solvent in green chemistry, has been showing great merits to the development of chemical industries, environmental engineering, etc (7,8). The SC-CO_2 process also exhibits potential applications of raw ingredients treatment in food industry (9). Due to different reaction matrices, the effect of SC-CO_2 treatment on Maillard reaction does not follow a consistent rule. Yalpani (10) demonstrated the high degrees of water soluble imine-linked, branched chitosan derivatives conversion in the SC-CO_2-treated mixtures of chitosan and glucose or malto-oligosaccharides, while the lactosylation of caseinmarcropeptide (CMP) or β-lactoglobulin (β-lg) and the formation of furosine were inhibited in a static SC-CO_2 system (11). Recently, Xu *et al.* (12) reported that the furfural, thiols, and disulfides, from ribose-cysteine system treated with SC-CO_2 at 140°C and 40 MPa over a pH range of 5.6-8.0, were enhanced comparing with those generated in conventional reaction systems. The parameters, affected Maillard reactions in SC-CO_2, not only include pH values, other important factors, such as temperature and water activity (Aw), also show great impacts on the products (3,4,19,22) and are worthy to be thoroughly studied before SC-CO_2 processing, a potential high-tech, is well applied in new food development.

The aim of this paper is to investigate the effect of high temperature on volatile products in ribose-cysteine mixtures heated under SC-CO_2 at 20 MPa for same periods as each conventional heating control to get the same browning.

Materials and Methods

Chemicals L-Cysteine hydrochloride (>98%) and sodium pyrophosphate tetrabasic (>99%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium pyrophosphate dibasic, alkane standard solution C_8-C_{20}, and tridecane were purchased from Sigma-Aldrich (St. Louis, MO, USA). D-Ribose was purchased from Amresco Inc. (Solon, OH, USA). The authentic aromatic chemicals were either purchased with the highest purity grade available from Sigma-Aldrich or obtained as gifts from flavor companies.

Preparation of model systems Aqueous 0.05 M ribose-cysteine solutions (pH 5.6 buffered with 0.2 M pyrophosphate) were prepared, and 100 mL aliquots were heated at different temperatures, either in airtight conventional chamber or at 20 MPa in SC-CO_2, in a CWYF-2 apparatus (Fig. 1,

*Corresponding author: Tel: +86-10-62737034; Fax: +86-10-62737986
E-mail: gyxcau@126.com
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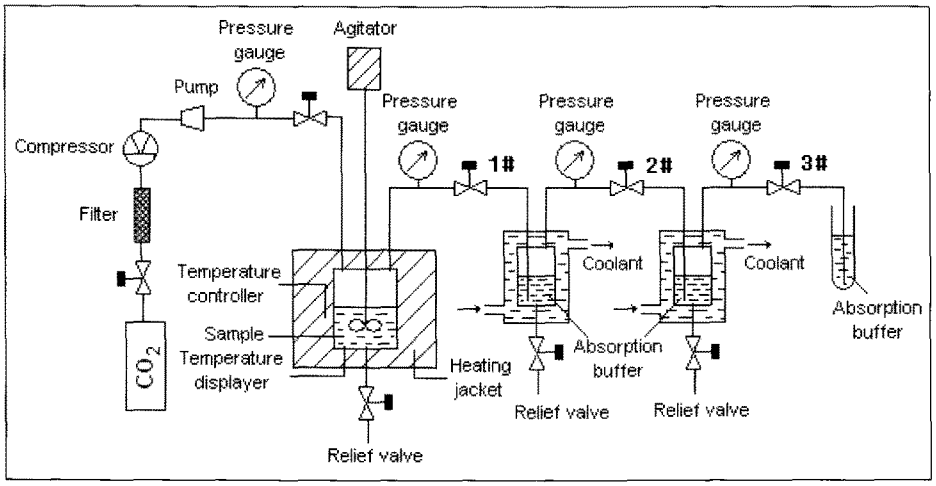

Fig. 1. Schematic diagram of the supercritical carbon dioxide (SC-CO_2) reaction system.

Hua'an Petroleum Scientific Instrument Co., Nantong, China). Model systems were cooled with cold 30%(v/v) ethanol (−3°C) immediately after the reaction, and the absorbance at 420 nm were measured. Matrices treated with SC-CO_2, at different temperatures, were heated for the same periods as corresponding conventional controls. At the end of the reaction under SC-CO_2, the chamber was then depressurized, allowing the CO_2, which was loaded with aromatic compounds, to be released sequentially into 2 absorption chambers, each containing 50 mL of the same pyrophosphate buffer used in the reaction mixture. The CO_2 was finally released through the outlet into another 50 mL of the same buffer to capture the remaining volatiles carried by the exhaust. The reaction mixture and the absorption buffers were collected separately and analyzed for volatile compounds. All the samples collected were stored below −18°C until use.

Volatile analysis The volatile compounds in reaction mixtures as well as the absorption buffers were analyzed by headspace solid-phase microextraction (HS-SPME) in tandem with gas chromatography coupled to mass spectrometry (GC-MS) following the procedures described previously (12). Absorption of volatiles with a DVB/CAR/PDMS (50/30 μm, 1 cm) fiber (Supelco Co., Bellefonte, PA, USA) was carried out at 60°C for 20 min. The oven temperature was initially set at 40°C, then heated to 60°C at 20°C/min, and held for 5 min, then raised to 250°C at 4°C/min, and kept at this temperature for 10 min. Approximate quantities of the volatiles adsorbed with the SPME fibre from each sample were estimated by comparing their peak areas, integrated using the total ion count (TIC) signals, with that of the tridecane internal standard (15.13 ng) using a response factor of 1. The concentrations of volatile compounds reported were the combined results obtained from the reaction mixture and the absorption buffers.

Statistical analysis The whole experiment was conducted in duplicate and all analyses were done at least in triplicate. The data were analyzed by one-way analysis of variance (ANOVA) using the Origin 7.5 software (OriginLab Corporation, Northampton, MA, USA). Significant differences of means were determined by the Tukey test.

Results and Discussion

As shown in Table 1, to get the same browning, long time is needed at lower temperatures. The relationship between the reaction time and temperature could fit in a linear equation: $\ln t = -11.356 \ln T + 60.772$ ($R^2 = 0.994$), where induction time is excluded, t is the reaction time (min) and T is temperature (°C). The absorbance values at 420 nm of the SC-CO_2-treated mixtures were about 20% smaller than those of corresponding controls, and there were no significant differences ($p > 0.05$) among the SC-CO_2-treated mixtures, except for that at 120°C with a little increase of browning. This could be attributed to the pressure-time integrated effect (20 MPa, 510 min) since high pressure had a positive effect on the formation of brown polymers with the extension of reaction time (13). The SC-CO_2 treatment could lead to a reversible pH decrease during the Maillard reaction (14) and the decrease of pH suppressed the formation of advanced products, therefore the absorbance at 420 nm declined.

More than 180 volatiles, which were dominated with sulfur compounds, have been identified in the Maillard reaction of heated ribose-cysteine model systems under various conventional conditions (15,16). Fifty-six compounds belonging to 8 classes were identified in the ribose-cysteine reaction products (Table 2). The total amount of all classes

Table 1. Temperature-time conditions applied to ribose-cysteine model system and absorbance data

Temperature (°C)	Time[1)] (min)	Absorbance[2)]	
		Control (CV)	SC-CO_2 (CV)
180	5	0.83 (2.6)^a	0.64 (2.2)^b
160	25	0.82 (1.7)^a	0.66 (1.5)^b
140	115	0.83 (0.5)^a	0.70 (0.1)^{bc}
120	510	0.84 (1.1)^a	0.75 (2.8)^c

[1)]Induction time was excluded.
[2)]Measured at 420 nm in a 10-fold dilution of the prepared model systems; Means with different letters are significantly different ($p < 0.05$).

Table 2. Volatile compounds generated from the ribose-cysteine model system[1] with or without SC-CO_2 at different temperatures[2]

LRI[3]	Compound	180°C		160°C		140°C		120°C		I.D.[4]
		Control	SC-CO_2	Control	SC-CO_2	Control	SC-CO_2	Control	SC-CO_2	
Polysulfur heterocyclic compound										
1153	3,5-Dimethyl-1,2,4-trithiolane (*E* or *Z*)	257.08	56.65	62.01	13.33	24.52	2.90	9.32	2.89	MS[21]
1160	3,5-Dimethyl-1,2,4-trithiolane (*E* or *Z*)	302.73	61.42	72.86	15.96	27.93	2.86	9.98	4.55	MS[21]
1185	1,2-Dithian-4-one	-	7.50	1.97	10.57	3.70	11.89	2.39	8.60	ms
1232	3-Methyl-1,2-dithian-4-one	-	4.32	-	7.80	-	22.63	3.34	31.39	MS[21]
1246	1,3,5-Trithiane	1.73	9.49	4.98	3.29	4.30	-	-	-	ms
1266	3-Methyl-1,2,4-trithiane	71.16	36.07	20.97	9.40	19.54	6.69	15.00	5.60	MS[21]
1413	3,6-Dimethyl-1,2,4,5-tetrathiacyclohexane (*E* or *Z*)	180.15	14.67	50.49	13.66	21.67	3.42	7.44	4.52	ms
	Total heterocyclics	812.85	190.12	213.27	74.02	110.75	27.83	45.53	26.16	
Thiol										
818	3-Mercapto-2-butanone	2.94	5.06	4.10	2.56	1.46	tr	-	tr	MS+LRI
869	2-Methyl-3-furanthiol	-	17.03	2.22	15.07	3.76	25.40	2.25	13.67	MS+LRI
904	3-Mercapto-2-pentanone	-	2.37	-	1.49	-	1.14	-	tr	MS+LRI
909	2-Mercapto-3-pentanone	1.66	3.66	0.62	1.26	-	0.76	-	tr	ms+LRI[18]
913	2-Furanmethanethiol	1.14	21.17	2.58	9.45	1.50	12.44	1.48	3.81	MS+LRI
980	3-Thiophenethiol	2.58	17.10	2.43	13.22	3.65	15.29	1.73	5.35	ms+LRI[18]
1066	2-Methyl-3-thiophenethiol	2.31	20.76	5.41	18.11	10.61	24.85	8.21	14.15	ms+LRI[19]
	Total thiols	10.63	87.14	17.37	61.16	20.97	80.05	13.68	37.58	
Disulfide										
1542	Bis(2-methyl-3-furyl)disulfide	3.06	11.39	2.74	19.90	4.68	34.45	2.49	33.95	MS+LRI
1643	2-Methyl-3-(2-furfuryldithio)furan	1.05	4.75	-	4.78	-	4.09	-	4.16	LRI[20]
1700	Bis(2-furfuryl)disulfide	0.73	0.77	0.64	0.64	1.38	tr	1.52	-	MS+LRI
1702	2,3-Dihydro-5-methyl-4-[(2-methyl-3-furyl)dithio]furan	-	17.99	-	22.15	1.48	24.91	1.30	14.40	ms
1745	2-Methyl-3-[(2-methyl-3-thienyl)dithio]furan	0.97	7.76	-	9.85	1.43	17.59	1.31	13.82	LRI[18]
1874	α-Dithiobisthiophene	-	3.02	-	3.64	-	1.97	-	-	ms
1921	3-Methyl-2-[2-thienyldithio]-thiophene	-	3.68	-	2.91	-	3.22	-	1.14	ms
	Total disulfides	5.81	49.37	3.38	63.86	8.97	86.54	6.63	67.52	
Thiophenone										
959	Dihydro-3-(2H)-thiophenone	2.45	3.94	2.10	1.50	0.37	0.69	-	1.03	MS+LRI
990	Dihydro-4(5)-methyl-3(2H)-thiophenone	-	2.59	-	-	-	-	-	-	ms
996	Dihydro-2-methyl-3(2H)-thiophenone	2.54	29.87	6.69	22.93	20.51	29.50	21.75	25.23	MS+LRI
1026	4,5-Dihydro-2,4-dimethyl-3(2H)-thiophenone (*E* or *Z*)	2.07	5.95	3.13	3.75	6.50	5.21	5.52	2.35	ms+LRI[18]
1171	α-Ethyl-3(2H)-thiophenone	1.95	7.53	3.35	3.29	4.18	3.81	2.79	3.03	ms
	Total thiophenones	9.01	49.88	15.27	31.47	31.56	39.22	30.05	31.64	
Thiophene										
773	2-Methylthiophene	1.97	2.54	4.61	2.32	10.61	2.88	-	4.57	ms+LRI[19]
1006	2-Thiophenecarboxaldehyde	6.33	20.96	4.08	10.14	5.52	12.27	2.28	7.31	ms
1068	2-Thienylmethanol	3.85	19.85	8.48	23.39	19.02	35.15	19.40	40.57	MS+LRI
1090	1-(3-Thienyl)-ethanone	46.00	43.89	33.29	19.74	35.11	26.81	30.24	16.37	ms
1095	1-(2-Thienyl)-ethanone	7.31	9.14	4.93	3.14	4.83	3.99	3.72	2.49	ms+LRI[18]
1128	3-Methyl-2-formylthiophene	18.37	43.34	17.28	30.07	20.73	35.83	15.71	28.05	ms+LRI[4]
1149	5-Methyl-2-thiophenecarboxaldehyde	3.08	5.89	6.98	2.35	6.38	1.96	4.05	1.11	MS+LRI
1189	2-Propionylthiophene	11.05	26.81	9.29	17.14	20.69	24.74	20.09	21.55	ms+LRI[4]
1194	2-Acetyl-3-methylthiophene	3.39	3.69	3.02	0.91	4.58	-	3.83	2.86	MS+LRI
1208	3-Ethyl-2-formylthiophene	6.35	25.20	5.62	19.67	11.90	34.61	8.64	33.85	ms+LRI[18]
1249	α-Dimethylformylthiophene	7.10	12.03	4.60	12.49	7.99	21.74	6.22	27.96	ms
1289	3-Acetyl-2,5-dimethylthiophene	14.75	21.93	19.21	8.45	8.51	4.19	2.05	1.09	ms
	Total thiophenes	129.55	235.28	121.39	149.81	155.89	204.17	116.23	187.76	
Fused bicyclic compound										
1197	2,3-Dihydro-6-methylthieno[2,3-*c*]furan	24.49	96.60	36.95	60.12	18.94	55.25	5.00	15.06	ms+LRI[18]
1215	α-Thienothiophene	5.00	8.57	7.97	3.79	5.16	1.01	1.06	-	ms

Table 2. Continued

LRI[3]	Compound	180°C		160°C		140°C		120°C		I.D.[4]
		Contr.	SC-CO_2	Contr.	SC-CO_2	Contr.	SC-CO_2	Contr.	SC-CO_2	
1220	Thieno[3,2-*b*]thiophene	45.78	140.75	52.19	68.74	56.06	45.41	22.13	21.85	ms+LRI[18]
1325	α-Dihydrothienothiophene	568.14	820.98	665.95	388.37	605.85	265.78	234.30	109.22	ms
1364	2-Methylthieno[2,3-*b*]thiophene	23.74	19.55	27.51	14.56	42.05	25.95	20.44	18.80	ms
1381	α-Methyldihydrothienothiophene	47.14	78.25	58.43	38.32	109.21	24.03	27.73	8.87	ms+LRI[18]
1418	α-Methyldihydrothienothiophene	13.39	87.15	27.21	57.79	43.08	37.83	15.31	21.65	ms+LRI[18]
1423	α-Methyldihydrothienothiophene	43.87	96.72	51.31	66.01	70.57	81.24	33.07	52.54	ms+LRI[18]
1477	α-Dimethyldihydrothienothiophene	11.27	17.94	21.92	16.95	69.96	27.61	33.57	18.59	ms
	Total fused bicyclics	782.82	1366.51	949.46	714.65	1020.89	564.13	392.62	266.59	
Pyrazine & Thiazole										
825	Methylpyrazine	9.09	0.85	4.26	-	2.82	-	2.30	-	MS+LRI
914	2,5-Dimethylpyrazine	2.31	+	-	-	-	-	-	-	MS+LRI
918	Ethylpyrazine	8.15	3.41	2.02	1.46	0.89	tr	1.19	-	MS+LRI
1022	2-Acetylthiazole	8.78	14.49	9.81	6.05	14.26	5.76	9.24	3.38	MS+LRI
1077	5-Ethyl-2,4-dimethylthiazole	2.25	7.29	0.43	3.49	2.03	2.96	1.57	2.52	ms+LRI[3]
	Total pyrazines and thiazoles	30.60	26.04	16.52	11.00	20.00	8.87	14.30	5.91	
Miscellaneous										
832	Furfural	3.51	7.74	1.41	6.07	1.54	14.16	1.50	4.19	MS+LRI
952	1-(2-Furyl)-2-propanone	1.55	2.81	1.77	2.11	1.95	5.02	2.38	4.17	ms
1009	1-(2-Furyl)-1-propanone	-	2.85	-	1.44	2.34	2.67	1.38	2.06	ms
1075	4-Hydroxy-5-methyl-3(2H)-furanone	2.95	5.91	6.20	13.69	4.09	4.73	0.89	1.73	MS+LRI
	Total miscellaneous	8.01	19.32	9.38	23.12	10.68	26.59	6.15	12.15	

[1]Each model system consisted of 0.05 M ribose and cysteine in 100 mL 0.2 M pyrophosphate buffer.
[2]Approximate quantities in headspace (ng/mL of mixture) given as means of independent experiments; tr, trace (<0.3 ng/mL of mixture); -, below detection limit (-0.07 ng/mL of mixture); +, present in small amounts and quantification confounded by adjacent peak.
[3]Linear retention index.
[4]I.D., identification method; MS+LRI, mass spectrum and LRI agree with those of authentic compound analysed in our laboratory; ms+LRI, mass spectrum agrees with the standard spectra in NIST 98 or Wiley 7n Mass Spectral Databases and the LRI near to the literature cited; MS, mass spectrum agrees with literature spectrum; ms, mass spectrum agrees with the standard spectra in NIST 98 or Wiley 7n Mass Spectral Databases. Numbers are reference number.

of compounds was higher at 180°C than at the other temperatures, and the relative abundance of each class of volatiles, generated from different treatments at each temperature, are shown in Fig. 2. The total fused bicyclic compounds were the main products in all the systems at different temperatures, excluding the control system at 180°C, where the total polysulfur heterocyclic compounds accounted for nearly 50% and was the most abundant volatile. These are broadly in consistence with data obtained for both aqueous and low-moisture systems based on cysteine and ribose (3,4,17). The total thiols, disulfides, thiophenones, and thiophenes were higher in SC-CO_2-treated mixtures at different temperatures whereas the total polysulfur heterocyclic compounds were smaller in SC-CO_2-treated mixtures than those in control systems. The relative abundance of fused bicyclic compounds in SC-CO_2-treated samples was also smaller as compared with those in controls except at 180°C. Regardless of the same extent of browning of the model system treated at different temperatures, a positive effect of high temperature on the volatiles formation was verified, and the volatiles, which were affected by the thermal and SC-CO_2 treatments, are discussed as follows.

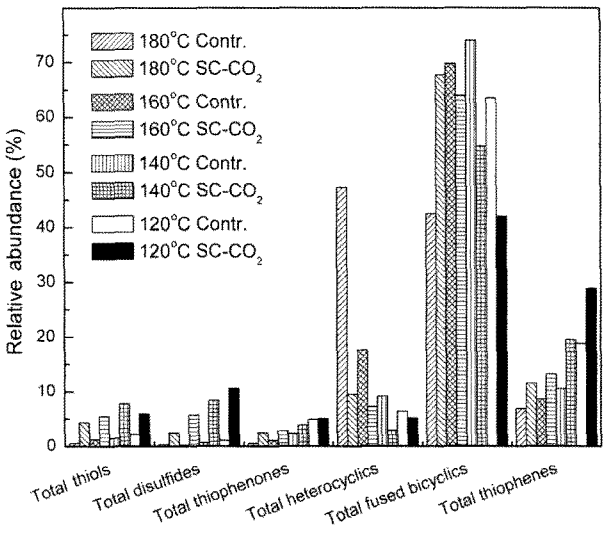

Fig. 2. Relative abundances of sulfur-containing classes of compounds for ribose-cysteine reaction products at different temperatures.

The concentrations of polysulfur heterocyclic compounds, the specific derivatives of cysteine degradations (15), were elevated with the increase of temperature in both process methods, and most of them in SC-CO_2-treated samples decreased significantly except for 1,2-dithian-4-one and 3-methyl-1,2-dithian-4-one. For example, the amount of 3,5-

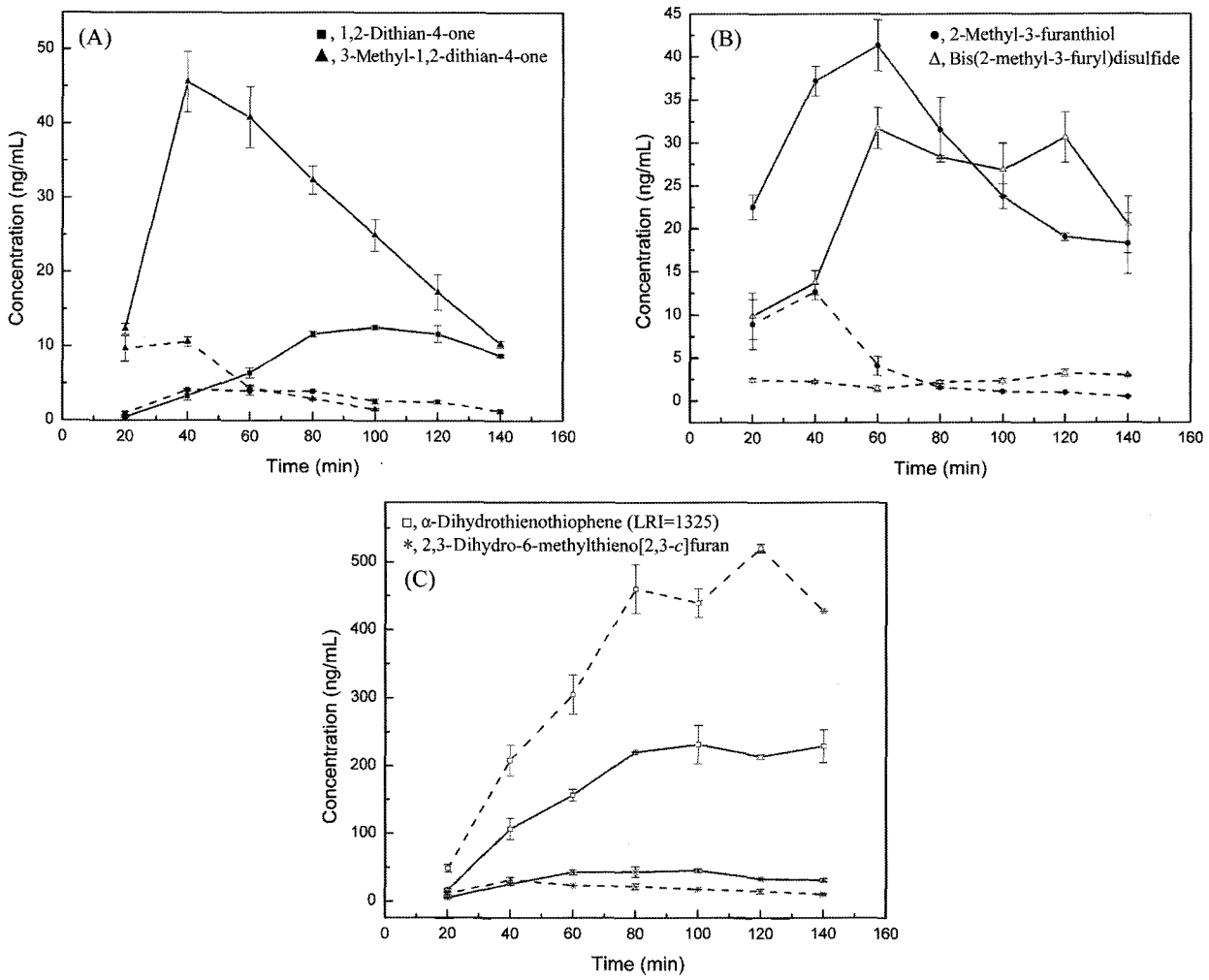

Fig. 3. Concentration changes of volatiles with the extension of reaction time (solid line, SC-CO_2 system; dash line, control system). Treatments were carried out in duplicate. Each sample underwent triplicate analyses with HS-SPME-GC-MS. Error bars represent mean±SD, n=2.

dimethyl-1,2,4-trithiolane (*E* or *Z*) generated at 180°C in conventional, were about 4, 10 and 30 times more than those at 160, 140, and 120°C, respectively. In comparison, the 3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*) generated in SC-CO_2-treated samples at 180°C was 4, 20, and 20 times more than those generated at 160, 140, and 120°C, respectively. The presence of SC-CO_2 led to a 65-90% decrease of 3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*) than those in conventional controls. 3,6-Dimethyl-1,2,4,5-tetrathiacyclohexane (*E* or *Z*) is another characteristic 4 sulfur atoms containing alicyclic volatile in cysteine degradation and cysteine-sugar reaction products. The concentration of 3,6-dimethyl-1,2,4,5-tetrathiacyclohexane (*E* or *Z*), detected at 180°C in control, was about 3, 8, and 24 times more than those at 160, 140, and 120°C, respectively. When in SC-CO_2, the 3,6-dimethyl-1,2,4,5-tetrathiacyclohexane (*E* or *Z*) at 180°C was about 1, 4, and 3 times more than those at 160, 140, and 120°C. The presence of SC-CO_2 decreased 93% of 3,6-dimethyl-1,2,4,5-tetrathiacyclohexane (*E* or *Z*) at 180°C. An inhibited Strecker degradation of cysteine in SC-CO_2 system was proposed to be partially responsible for the reduction of polysulfur heterocyclic compounds (12).

The concentrations of 1,2-dithian-4-one and 3-methyl-1,2-dithian-4-one were always higher in SC-CO_2-treated samples at different temperatures. Different from 3 sulfur atoms containing alicyclic thiolanes, the formation of carbonyl containing dithianes seemed to favor at lower temperatures, especially for 3-methyl-1,2-dithian-4-one. In conventional heated mixtures, there was no presence of 3-methyl-1,2-dithian-4-one at 180, 160, and 140°C, and only 3.34 ng/mL was detected at 120°C. When the ribose-cysteine system heated in SC-CO_2, the concentration of 3-methyl-1,2-dithian-4-one at 120°C was about 7, 4, and 1.5 times more than those generated at 180, 160, and 140°C respectively. The high temperature and long time heating could degrade the polysulfur heterocyclic compounds. In the case of heating at 140°C, the accumulation of 3-methyl-1,2-dithian-4-one firstly increased to a peak value and then decreased along the extended reaction time (Fig. 3A). The balance between the accumulation and degradation of polysulfur heterocyclic compounds at different temperatures resulted in the different concentrations as listed in Table 2. The formation mechanism of 3-methyl-1,2-dithian-4-one involved was suggested to be similar to 2-methyl-4,5-dihydro-3(2H)-thiophenone (22). In SC-CO_2, the higher concentrations of 4-hydroxy-5-methyl-3(2H)-furanone at

different temperatures could account for the high levels of 3-methyl-1,2-dithian-4-one.

Thiols were a category key meat flavor volatiles characterized with low thresholds and sensitive to temperatures. The highest level of total thiols in SC-CO_2-treated matrices was found to be 87.14 ng/mL at 180°C, whereas it was 20.97 ng/mL at 140°C in control. For the same browning ribose-cysteine mixtures, the effect of temperature did not figure out in a relative simple trend, and the most abundant representatives of thiols were 2-methyl-3-furanthiol, 2-methyl-3-thiophenethiol, and 3-thiophenethiol. These 3 compounds were identified at much lower levels in conventional controls and got their highest concentrations at 140°C, while 3-mercapto-2-butanone and 2-furanmethanethiol got their peak values at 160°C. In SC-CO_2, 2-methyl-3-furanthiol and 2-methyl-3-thiophenethiol also got their peak concentrations at 140°C and the maximum concentrations of other thiols were at 180°C. The relative high concentrations of mercapto substituted heterocyclic compounds present at high temperatures could be attributed to the immediately interaction between hydrogen sulfide and 4-hydroxy-5-methyl-3(2H)-furanone, which was more concentrated in SC-CO_2-treated mixtures than in controls. 2-Furanmethanethiol possesses an intense roasted and coffee-like odor and is derived from hydrogen sulfide and furfural or 2-furanmethanol (15). 2-Furanmethanethiol reached the highest concentration at 180°C (21.17 ng/mL) in SC-CO_2-treated matrix and it could be explained with the higher level of furfural and the immediately interaction between furfural and hydrogen sulfide at high temperature.

Disulfides, dimers of thiols, are also higher in SC-CO_2-treated samples and could be due to their high amount of monomers. Bis(2-methyl-3-furyl)disulfide, 2,3-dihydro-5-methyl-4[(2-methyl-3-furyl)dithio]furan, and 2-methyl-3-[(2-methyl-3-thienyl)dithio]furan were the 3 representatives among the identified disulfides and there was no dithiobisthiophene and 3-methyl-2-[2-thienyldithio] thiophene detected in conventional thermal treated mixtures. The most concentrated disulfides in the same browning reaction matrices appeared at 140°C in 2 reaction systems. The peak values of disulfides, in different temperature heated products, could be the integrated effects of temperature and reaction times as bis(2-methyl-3-furyl)disulfide firstly increased to a peak value, and then decreased with the extension of reaction time at 140°C (Fig. 3B).

Thiophenes (including thiophenones) were another major sulfur containing volatiles from aqueous ribose-cysteine Maillard reaction products (17). The thiophene derivatives appeared to be related with the pathway by which the individual compound is formed. In case of 2-thienylmethanol, a maximum concentration was reached at 120°C in both systems. However, 1-(3-thienyl)-ethanone favored at high temperatures. Dihydro-2-methyl-3(2H)-thiophenone got its top accumulation at 120°C in conventional control. In comparison, there was no apparent effect of high temperature on its accumulation in SC-CO_2. Interestingly, with the participation of SC-CO_2, most of the carbonyl substituted thiophenes were more concentrated than those in corresponding controls, such as 2-thiophenecarboxaldehyde and 3-methyl-2-thiophenecarboxaldehyde. The carbon skeleton of thiophenes was derived from 4-hydroxy-5-methyl-3(2H)-furanone and the substituted thiophenes were mostly derived from the aldol condensation of fragments from 4-hydroxy-5-methyl-3(2H)-furanone and cysteine (18), the aforementioned higher amount of 4-hydroxy-5-methyl-3(2H)-furanone in the SC-CO_2-treated reaction mixtures could account for the high level of carbonyl substituted thiophenes in SC-CO_2-treated mixtures.

Thienothiophenes and 2,3-dihydro-6-methylthieno[2,3-*c*]furan (kahweofuran) are the largest class of fused bicyclic volatiles in aqueous ribose-cysteine reaction mixtures. In general, SC-CO_2 inhibited the accumulation of total thienothiophenes except for those detected at 180°C. In conventional reaction mixtures, most of the methyl-thienothiophenes got their maximum concentrations at 140°C, while in SC-CO_2, the top values were found at 180°C except for 2-methylthieno[2,3-*b*]thiophene. The dihydrothienothiophene, which linear retention index (LRI) is 1,325, is the most abundant volatile detected in cysteine containing model systems. It was readily formed at high temperatures and inhibited in SC-CO_2-treated samples, except at 180°C. The formation and decay rates of the dihydrothienothiophene at 140°C were slower in SC-CO_2 than in conventional system (Fig. 3C). Kahweofuran was found in coffee and bread volatiles with roasted and smoky notes in high dilutions (23) and also detected in ribose-cysteine reaction products (5,24). Under SC-CO_2, there always present a higher concentration of kahweofuran and the maximum was at 180°C, while in conventional systems, it reached the maximum concentration at 160°C. At 140°C, the reaction times, required for kahweofuran to reach the maximum values in conventional and SC-CO_2 systems, were not the same and dropped behind when treated with SC-CO_2. The integrated effect of high temperature and high pressure/SC-CO_2 on the generation of fused bicyclic compounds is still to be elucidated.

Pyrazines and thiazoles, important nitrogen containing heterocyclic volatiles, had been widely investigated in sugar-amino acid model systems under atmospheric conditions (25). Higher amount of pyrazines and thiazoles were found in control samples than those in SC-CO_2-treated mixtures, except for 5-ethyl-2,4-dimethylthiazole. The concentrations of pyrazines increased with the elevation of temperatures in both systems. There were even no 2,5-dimethylpyrazine identified in reaction mixtures heated below 160°C. The inhibited Strecker degradation of cysteine in SC-CO_2 could account for relative low levels of pyrazines. 2-Acetylthiazole has been reported in a number of cooked foods, and characterized with roasted popcorn and nutty notes (23). The highest concentration of 2-acetylthiazole in conventional reaction products was observed at 140°C, while the top value in SC-CO_2-treated mixtures was at 180°C, and there was no significant differences ($p>0.05$) between the 2 values. 2-Acetylthiazole was generated with mercaptoiminenol, produced by the Strecker degradation of cysteine and 2-oxopropanal, undergoing ring closure by necleophilic attack of the thiol group on the double bond of the enol (16). Although the Strecker degradation is favored at high temperatures, and inhibited in SC-CO_2 system, the exact mechanism concerning high accumulation of 2-acetylthiazole at 180°C in SC-CO_2 needs to be further explored.

In conclusion, high temperature generally promoted the

formation of noncarbonyl polysulfur heterocyclic compounds and thienothiophenes and its effect on volatiles was apparently related with the pathway by which the individual compound is formed. However, the characteristic meat note volatiles, especially thiols, disulfides, and kahweofuran, were favorably generated in SC-CO_2. In addition, the effect of SC-CO_2 treatment on Maillard reaction over 180°C needs more investigations.

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