Food Chemistry 232 (2017) 135–144

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Roles of different initial Maillard intermediates and pathways in meat flavor formation for cysteine-xylose-glycine model reaction systems

Li Hou [a], Jianchun Xie [a,*], Jian Zhao [a], Mengyao Zhao [a], Mengdie Fan [a], Qunfei Xiao [a], Jingjing Liang [a], Feng Chen [b]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Laboratory for Food Quality and Safety, Beijing Technology and Business University (BTBU), Beijing 100048, China
[b] Department of Food, Nutrition and Packaging Science, Clemson University, Clemson, SC 29634, USA

ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form 18 March 2017
Accepted 23 March 2017
Available online 24 March 2017

Keywords:
Meat flavor
Maillard reaction
Intermediate
Amadori
Thiazolidine
Cysteine
Glycine
Xylose

ABSTRACT

To explore initial Maillard reaction pathways and mechanisms for maximal formation of meaty flavors in heated cysteine-xylose-glycine systems, model reactions with synthesized initial Maillard intermediates, Gly-Amadori, TTCA (2-threityl-thiazolidine-4-carboxylic acids) and Cys-Amadori, were investigated. Relative relativities were characterized by spectrophotometrically monitoring the development of colorless degradation intermediates and browning reaction products. Aroma compounds formed were determined by solid-phase microextraction combined with GC–MS and GC-olfactometry. Gly-Amadori showed the fastest reaction followed by Cys-Amadori then TTCA. Free glycine accelerated reaction of TTCA, whereas cysteine inhibited that of Gly-Amadori due to association forming relatively stable thiazolidines. Cys-Amadori/Gly had the highest reactivity in development of both meaty flavors and brown products. TTCA/Gly favored yielding meaty flavors, whereas Gly-Amadori/Cys favored generation of brown products. Conclusively, initial formation of TTCA and pathway involving TTCA with glycine were more applicable to efficiently produce processed-meat flavorings in a cysteine-xylose-glycine system.

1. Introduction

The Maillard reaction plays an important role in the formation of food color and flavor during thermal processing (Chen, Jin, & Chen, 2005; Leiva, Naranjo, & Malec, 2017; Yu, Seow, Ong, & Zhou, 2017). It involves a series of complex chemical reactions that occurs between carbonyl compounds and amino compounds, such as reducing sugars and amino acids (Jalbout, Shipar, & Navarro, 2007). According to the Hodge scheme (Hodge, 1953), the Amadori rearrangement can take place between the aldose sugars and amino acids in the initial (early) stage of the Maillard reaction, leading to the formation of Amadori rearrangement products, which are the primary reaction intermediates that play a critical role in further development of the final Maillard reaction products (Davidek, Kraehenbuehl, Devaud, Robert, & Blank, 2005; Jalbout et al., 2007; Leiva et al., 2017; Troise, Buonanno, Fiore, Monti, & Fogliano, 2016). The Amadori rearrangement products can be decomposed into α-dicarbonyls with various chain lengths, such as 3-deoxyhexos-2-ulose, 1-deoxy-2,3-hexodiulose, 2-oxopropanal, butane-2,3-dione, and glyoxal (Mottram & Elmore, 2010; Wang & Ho, 2010). The very reactive α-dicarbonyls will initiate a cascade of further reactions, resulting in a complex mixture composed of various Maillard reaction products, in particular, the volatile flavor compounds and the brown pigments (Desclaux, Malik, Winkel, Pyle, & Mottram, 2006; Glomb & Tschirnich, 2001; Leiva et al., 2017; Mottram & Elmore, 2010).

Cysteine has been well known as a precursor of sulfur-containing meaty flavors (Cerny, 2007; Cerny & Davidek, 2003; Cerny & Guntz-Dubini, 2013; Hofmann & Schieberle, 1995, 1997). For a simple model reaction of cysteine and reducing sugars such as xylose, as illustrated in Fig. 1, the relatively stable cyclic 2-threityl-thiazolidine-4-carboxylic acid (TTCA), rather than the Amadori rearrangement product of cysteine (Cys-Amadori), is primarily formed in the early stage of the Maillard reaction, which circumvents the development of meaty flavors from cysteine (de Roos, 1992; de Roos, Wolswinkel, & Sipma, 2005). However, under heating, TTCA can be reversibly converted into Cys-Amadori (Fig. 1). Glycine is the simplest amino acid usually used in preparation of processed-meat flavorings. Regarding a complex reaction system composed of cysteine, xylose, and glycine (cysteine-xylose-glycine), in the initial stage of the Maillard reaction, both cysteine

* Corresponding author.
E-mail address: xjchun@th.btbu.edu.cn (J. Xie).

http://dx.doi.org/10.1016/j.foodchem.2017.03.133
0308-8146/

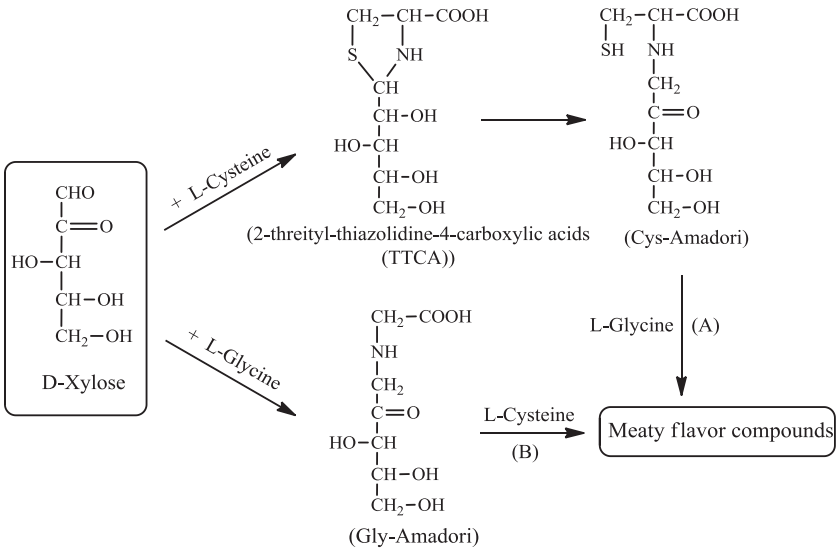

Fig. 1. The two major pathways to develop meaty flavors in a complex meat-like model reaction system of cysteine-xylose-glycine in terms of the intermediates formed in the initial stage of the Maillard reaction. Note. There are tautomers, or cyclic structures for the Amadori compounds, while only the open-chain forms are shown.

and glycine can react with xylose, forming three possible initial stage intermediate products, TTCA, Cys-Amadori, and Gly-Amadori (Amadori rearrangement product of glycine) (see Fig. 1). With regards to the three initial intermediate products, two main pathways have been suggested to develop meat-like flavors (Fig. 1): (A) the reaction of the degradation products of Cys-Amadori with glycine, where the former is usually being converted from TTCA; (B) the reaction of the degradation products of Gly-Amadori with cysteine. The complication is that *Path A* can be promoted by glycine, whereas *Path B* can be inhibited by cysteine (de Roos, 1992; de Roos et al., 2005). In our previous research to investigate the preparation technology of processed-meat flavorings using enzymatic hydrolyzate of meat, it was found the thermal reaction composed of glycine and Cys-Amadori (or TTCA) could produce more sulfur-containing meaty flavors *via Path A* than that composed of cysteine and Gly-Amadori via *Path B* (Gong et al., 2016). However, it is still unclear what underlying reactive differences of the individual initial intermediates and the aforementioned pathways, and mechanisms lead to such a result.

Therefore, in this study, the reaction models of Gly-Amadori with cysteine (Gly-Amadori/Cys), Cys-Amadori with glycine (Cys-Amadori/Gly), and TTCA with glycine (TTCA/Gly), together with Gly-Amadori alone, Cys-Amadori alone, and TTCA alone, were designed and investigated. Through the comparison of rate and degree of reaction and amount of meaty compounds produced in the final reaction solutions, it was expected to reveal roles of the different initial intermediate products and the pathways, and relevant mechanisms for the development of meaty flavors in a complex reaction system of cysteine-xylose-glycine. In addition, the work is also hoped to provide an effective means for efficient preparation of the processed-meat flavorings.

2. Experimental

2.1. Reagents and chemicals

L-Cysteine (99%), D-xylose (99%), and L-glycine (99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). The *n*-alkanes (C_6-C_{27}), and standards, including 2-methylthiophene (99%), 1-(2-thienyl)-1-propanone (98%), 1-(3-thienyl)-ethanone (99%), 2,3-dihydro-5-methylthiophene (98%), 2,3-dimethylpyrazine (98%), 2,3-dimethylthiophene (99%), 2,4,5-trimethylthiazole (98%), 2,4-dimethylthiazole (98%), 2,5-dimethylpyrazine (97%), 2,5-dimethylthiophene (99%), 2,5-thiophenedicarboxaldehyde (98%), 2-acetyl-3-methylthiophene (97%), 2-acetylthiazole (98%), 2-ethyl-4-methylthiazole (97%), 2-ethyl-5-propylthiophene (98%), 2-ethylthiazole (98%), 2-ethylthiophene (99%), 2-furfurylthiol (97%), 2-methyl-3-furanthiol (97%), 2-methylthiazole (98%), 2-thiophenecarboxaldehyde (99%), 2-thiophenemethanethiol (98%), 3-mercapto-2-butanone (97%), 3-mercapto-2-pentanone (98%), 3-methyl-2-thiophenecarboxaldehyde (99%), 3-methylpyridine (97%), 3-thiophenethiol (98%), 4,5-dimethylthiazole (99%), 4-methylthiazole (98%), 5-methyl-2-thiophenecarboxaldehyde (98%), benzothiazole (99%), bis(2-furfuryl) disulfide (97%), bis(2-methyl-3-furyl)disulfide (98%), dihydro-2-methyl-3(2*H*)-furanone (98%), dihydro-2-methyl-3(2*H*)-thiophenone (99%), furfural (99%), pyrrole (98%), thiazole (99%), trimethylpyrazine (98%), and thiophene (98%), which were used to identify the compounds in GC–MS and GC-olfactometry (GC-O) analysis, were purchased from J&K Chemical Ltd. (Beijing, China). The other chemicals used in this study were all of analytical grade.

2.2. Syntheses of Gly-Amadori, Cys-Amadori, and TTCA

Gly-Amadori (N-(1-deoxy-D-xylulos-1-yl-L-glycine) was prepared according to Hao et al. (2007). Cys-Amadori (N-(1-deoxy-D-xylulos-1-yl)-L-cysteine) and TTCA (2-threityl-thiazolidine-4-carboxylic acids) were prepared as described by De Roos et al. (2005). Particularly, the reaction mixtures of cysteine and xylose at 50 °C for 1 h were selected to isolate TTCA. To purify the aforementioned three intermediate products, repeated separations were conducted using a preparative column (35 × 1.6 cm) packed with Bio-Rad AG 50 W-X4 (H^+) cation exchange resin of 200–400 mesh, on an MD-99 automatic liquid chromatographic system (Shanghai Qingpu Huxi Instruments Co., Shanghai, China) with UV monitoring at 220 nm. The column was at first washed with demineralized water, and then eluted gradually with 0.3 M ammonia solution. Based on the UV chromatogram, the fractions were collected, pooled, and then lyophilized.

Purities (in relative peak area) of the prepared intermediates, i.e., Gly-Amadori, Cys-Amadori, and TTCA, were ≥99%, ≥98%, and ≥99%, respectively, being analyzed by high-performance liquid chromatography and evaporative light scattering detection (HPLC-ELSD). An Agilent 1100 HPLC system (Agilent Technologies,

Table 1
The model reaction systems.

Systems	Amounts (mmol)				
	Cys-Amadori	Gly-Amadori	[1]TTCA	Cysteine	Glycine
Gly-Amadori/Cys		0.15		0.15	
TTCA/Gly			0.15		0.15
Cys-Amadori/Gly	0.15				0.15
Cys-Amadori alone	0.15				
TTCA alone			0.15		
Gly-Amadori alone		0.15			

[1] TTCA, 2-threityl-thiazolidine-4-carboxylic acid.

Santa Clara, CA) coupled with a SEDEX 75 ELSD detector (Sedere, Alfortville, France) was used in the HPLC-ELSD analysis. The HPLC conditions were the same as those described in the following Section 2.5 for HPLC-MS analysis. The ELSD evaporation tube temperature was set at 40 °C. The air compressor for the evaporation tube was set at 3.5 mL/min.

In addition, for the above mentioned three synthesized intermediates, their mass spectra (MS) were determined by HPLC-MS according to the following Section 2.5, while their ^{13}C NMR spectra (D_2O) were determined by Bruker 300 MHz. The MS and NMR data were presented in Table S1, which agreed with those reported in the literature (Davidek et al., 2005; De Roos et al., 2005).

2.3. Model reactions

The reaction systems and the used amounts of reagents are presented in Table 1. In 15-mL pressure glass vials (Beijing Synthware Glass Inc., China), the reagents were dissolved in 5 mL of sodium phosphate buffer solution (pH 5.5, 0.2 M). The vials were sealed under ambient air, and then heated at 140 °C while stirring for 5, 10, 15, 20, 25 or 30 min on a Parallel Synthesis Poly-block 4 system (HEL, Borehamwood, UK). At the end of the respective reaction time, the vials were removed and cooled immediately by a stream of tap water. Three replicates were performed and subjected to the following analyses.

2.4. Measurement of pH and UV/Vis absorbance

A UV 2700 spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan) and a PHSJ-5 pH meter (Inesa instrument Co., Shanghai, China) were used. UV/Vis absorbance was measured at 294 nm and 420 nm, respectively, while the samples without the heat treatment were used as blanks. When an appropriate dilution of a sample was performed, the measured values were converted to those of the original reaction solutions.

2.5. High-performance liquid chromatography and mass spectrometry (HPLC-MS)

An LCQ-DECA XP MAX HPLC-MS system equipped with a high performance liquid chromatography and a trap tandem mass spectrometer (Thermo-Electron Company, San Jose, CA) was used. The detection was performed in the positive electrospray ionization (ESI) mode. Capillary temperature was 350 °C. Capillary voltage was 35 V. Ion source voltage was 5 kV. Ion source current was 80 μA. Sheath gas flow rate and auxiliary gas flow rate were 40 arb and 10 arb, respectively. MS detection was in full scan mode over the range of 50–800 amu, or in multiple reaction monitoring (MRM) mode. The MRM mode was performed on m/z 254, 208, and 311, with 35% normalized collision energy and nitrogen as a collision gas, which were respectively to identify the synthesized Cys-Amadori (or TTCA) and Gly-Amadori, and the formed G-TTCA in the Gly-Amadori/Cys reaction solution. Data were acquired with Xcalibur software system (Thermo-Electron Company, San Jose, CA).

According to Cao et al. (2016), an Xbridge Amide column (4.6 mm × 150 mm, 3.5 μm; Waters Co., Milford, MA) was used in the HPLC separation. Composition of the mobile phase was acetonitrile and ammonium formate (10 mM, pH 6). The mobile phase was eluted in a linear gradient, 0–5 min, 95% → 75% of acetonitrile (v/v); 5–25 min, 75% → 65% of acetonitrile (v/v). After the elution, the system was restored to the initial conditions for 5 min. Flow rate of the mobile phase was 0.5 mL/min. Column temperature was 25 °C. The injection volume of sample was 5 μL.

2.6. Solid-phase microextraction (SPME)

Volatile compounds in the final reaction solutions were extracted by solid-phase microextraction (SPME). A manual SPME holder together with 15-mL vials, Teflon-lined septa, and a 50/35 μm Carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) fiber (Supelco Inc., Bellefonte, PA) was used. In a 15-mL vial, 1 mL of the reaction solution and 4 mL of the phosphate buffer (pH 5.5, 0.2 M) were placed. After pre-equilibration at 50 °C for 10 min, the volatile compounds were adsorbed at 50 °C with the fiber in the headspace of the vial for 20 min, while being agitated by an electromagnetic stirrer. The adsorbed fiber was directly introduced into a GC injector for the subsequent GC–MS or GC-O analysis.

2.7. Gas chromatography and mass spectrometry (GC–MS)

An Agilent 7890A GC coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA) was used. The separation was conducted on an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) and a DB-Wax (30 m × 0.25 mm × 0.25 μm) capillary column (Agilent Technologies, Santa Clara, CA). The carrier gas was helium at 1 mL/min. For the HP-5 MS column, the initial oven temperature was 40 °C, raised to 60 °C at 5 °C / min; raised to 150 °C at 3 °C /min; and finally raised to 250 °C at 10 °C /min. For the DB-Wax column, the initial oven temperature was 40 °C, held for 2 min; raised to 80 °C at 3 °C/min, held for 3 min; raised to 120 °C at 4 °C/min, held for 2 min; and finally raised to 230 °C at 10 °C /min, held for 2 min.

The fiber was desorbed at 250 °C for 3 min in a splitless mode, and MS was detected with no solvent delay. The mass detector was operated at 150 °C in electron impact mode at 70 eV. The ion source temperature was 230 °C. The transfer line temperature was 280 °C. The chromatograms were recorded by monitoring the total ion current from m/z 40–450 mass range.

2.8. Gas chromatograph and olfactometry (GC-O)

An Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with an FID detector and a DATU 2000 high-resolution olfactometry system (DATU Inc., Geneva, NY) was

used. The column was DB-Wax (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Santa Clara, CA). The carrier gas was nitrogen at 1.0 mL/min. The programmed oven temperatures were the same as those for the DB-Wax column in the above GC–MS analysis. The GC effluent to the odor port was enclosed with a stream of humidified air at 16 L/min and transferred by one length of stainless steel tube (10 mm i.d.) to the Teflon detection cone.

Three trained panelists performed the GC-O analysis. The dilutions (1–256) were achieved through a stepwise increase of the injector split ratio (2:1, 4:1, 8:1, . . . 128:1) and at 128:1 with a contraction of the SPME fiber length exposed (1/2 of full exposure). The odor characteristics detected by the panelists were recorded and each odorant was finally assigned a FD (flavor dilution) factor representing the highest dilution that could just be perceived. For those still detectable at the dilution ratio of 256, the FD factors were recorded as FD ≥ 256, which were defined to be potent odorants. Retention times of the odorants were converted into retention index (RI) values relative to the series of *n*-alkanes (C_6-C_{27}).

2.9. Identification of the volatile flavor compounds

The identification of the volatile flavor compounds was based on mass spectra in GC–MS, retention indices (RI) relative to C_6-C_{27} *n*-alkanes in both GC–MS and GC-O analyses, odor characteristics detected by GC-O, and the comparison of the above parameters with those of the available standards listed in Section 2.1. In GC–MS analyses, both a polar column (DB-Wax) and a non-polar column (HP-5) were employed, while the mass spectra of the peaks and the corresponding RI values on both columns were used for the identification. For mass spectra, compounds were identified according to the NIST 2011 mass spectral library together with manual interpretation.

2.10. Statistic analysis

All the results were the averages of three replicates. The figures were plotted with Chemdraw 7.0 and Microsoft Excel 2010. Differences between means were handled by one-way ANOVA with Duncan's multiple range tests using SPSS 19.0 for windows (SPSS Inc., Chicago, IL). A *p*-level less than 0.05 was defined as significantly different.

3. Results and discussion

3.1. Rate and degree of reaction in the model systems

The previous research work on studying the mechanism of formation of meat-like aromas through the Maillard reactions usually used simple model systems composed of a single amino acid of free cysteine and reducing sugars (Cerny & Davidek, 2003; Mottram & Nobrega, 2002; Yang et al., 2015). In this study, a complex model reaction system containing cysteine, xylose as well as glycine was investigated. In addition to the free amino acids, the three possible initial intermediate products (i.e., Cys-Amadori, Gly-Amadori, and TTCA) formed in the reaction system were chosen as the reactants. The reactions were conducted in sealed vials under continuous stirring, which was simulated to the way of common pressure cooking of meat. According to Lerici, Barbanti, Manzano, and Cherubin (1990), the colorless degradation of compounds formed during the intermediate stage of the Maillard reaction were measured by UV/Vis absorbance at 294 nm, while the brown products formed during the final stage of the Maillard reaction were measured by UV/Vis absorbance at 420 nm. The degree of depletion of the amino group at the early stage along with the formation of organic acids in the final stage was indicated by the decrease of pH value of the reaction solution (Brands & Van Boekel, 2002; Liu, Yang, Jin, Hsu, & Chen, 2008; Martins, Marcelis, & van Boekel, 2003).

As a result, changes of UV/Vis absorbance and pH values for the six reaction systems *vs.* reaction time are shown in Fig. 2. In general, an accumulation of the colorless degradation products reflected by the 294 nm absorbance (Fig. 2a) and the browning products reflected by the 420 nm absorbance (Fig. 2b), and a decrease of pH, occurred as a function of time (Fig. 2c). The four model systems that contained an Amadori compound (i.e., Cys-Amadori/Gly, Cys-Amadori alone, Gly-Amadori/Cys, and Gly-Amadori alone) exhibited similar reaction profiles (Fig. 2a-b), except the Gly-Amadori alone system that showed its maximal absorbance at 294 nm at 5 min and then decreased markedly. These delicate discrepancies in spectrophotometric observations were ascribed to the differences in rates of reaction of the individual systems, which are discussed as follows.

With regards to the curves (i.e., absorbance at 294 nm and 420 nm, and pH change) of the three initial intermediates alone, Gly-Amadori tended to have the fastest degradation among the three individual intermediates monitored, followed by Cys-Amadori, and then TTCA. The TTCA intermediate had the lowest rate and degree of degradation among the three intermediates because of the existence of its relatively stable thiazolidine structure (De Roos, 1992; De Roos et al., 2005). As for the Gly-Amadori intermediate, the aforementioned exceptional occurrence of maximal value at 294 nm absorbance was ascribed to its high rate of reaction, which was considered to result from the rapid formation of the colorless degradation products at first and then the rapid transformation of the colorless degradation products to the brown products subsequently (see Fig. 2a-b). In addition, also for the Gly-Amadori alone system, with the decrease of absorbance at 294 nm after 5 min, simultaneously, an increase of absorbance at 420 nm was observed (see Fig. 2a-b,) indicating a large proportion of the colorless degradation products were precursors of the brown products.

As shown in Fig. 2, the effect of inclusion of glycine or cysteine on the degradation rate of the three individual intermediates was varied. The reaction solution of the TTCA/Gly system tended to have greater absorbances at 294 nm and 420 nm and lower pH values than the corresponding values of the TTCA alone system as a result of the promotion effect of glycine. In fact, glycine has been observed to be able to accelerate the Maillard reaction between cysteine and reducing sugars to develop meaty flavors, where the acceleration is considered general-acid-catalyzed (De Roos et al., 2005; Zeng, Li, He, Qin, & Chen, 2012). According to Fig. 1, the catalytic effect of glycine is hypothesized to be mainly in the conversion step from the TTCA to Cys-Amadori. Moreover, compared to the Cys-Amadori alone system, at 420 nm absorbance some increase was also observed in the Cys-Amadori/Gly system, although their absorbances at 294 nm and pH values were almost the same. This increase at 420 nm absorbance was mainly due to the participation of added glycine in the reaction with the colorless degradation products of Cys-Amadori in the Cys-Amadori/Gly system.

Particularly, compared to the Gly-Amadori alone system, absorbance values at 294 nm and 420 nm of the Gly-Amadori/Cys system were markedly decreased (Fig. 2a, b), and the pH values of the latter were also observed much lower than the corresponding values of the former (Fig. 2c). These results suggested cysteine had inhibited the degradation reaction of Gly-Amadori (Huang et al., 2012). It has been reported that cysteine is able to react with the Amadori products, deoxyosones, or other intermediate products in the Maillard reaction solution to form relatively stable cyclic compounds (Yaylayan & Huyghues-Despointes, 1994). For instance, a relatively stable cyclic compound of 1,4-thiazane,

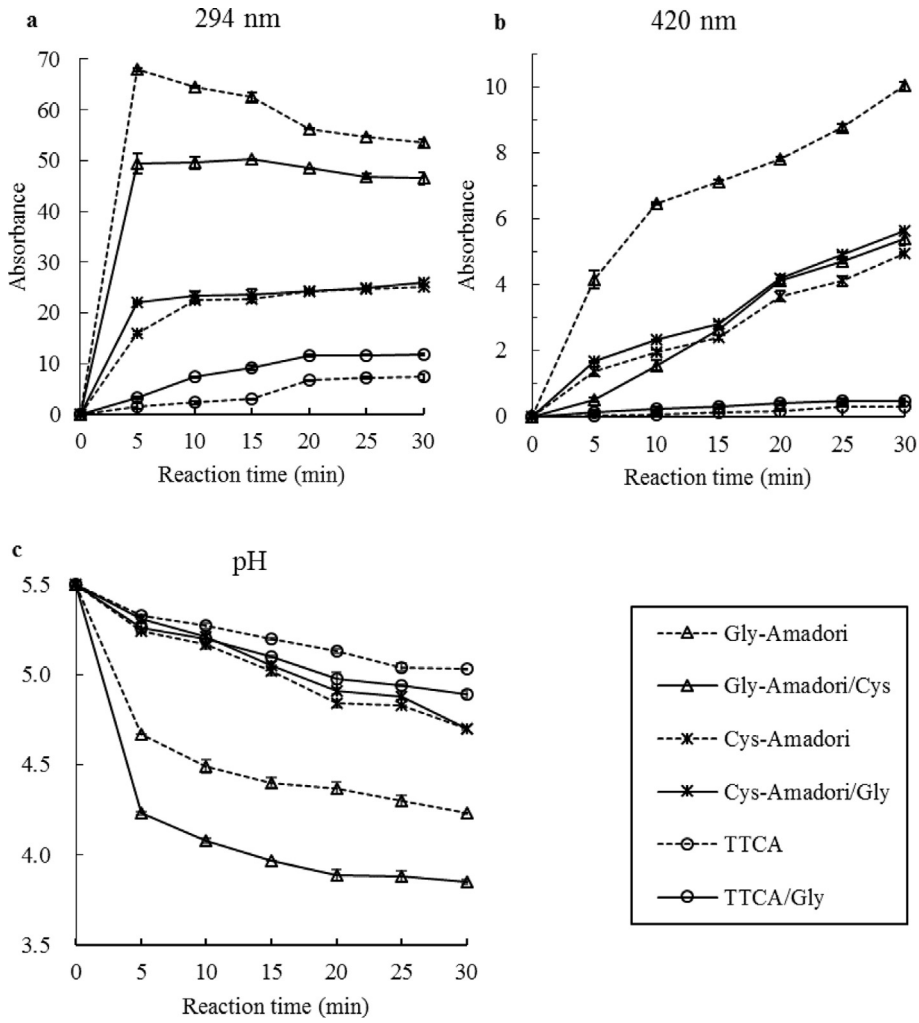

Fig. 2. Changes of (a) 294 nm and (b) 420 nm UV/Vis absorbance and (c) pH of the reaction solutions for the systems of Cys-Amadori/Gly, Gly-Amadori/Cys, TTCA/Gly, Cys-Amadori alone, Gly-Amadori alone, and TTCA alone with reaction time. The data used in plotting the figures were means ± standard deviations (n = 3).

which was formed from 1-deoxyosone and cysteine, had been characterized from the heated aqueous solution of a Gly-Amadori (1-deoxymaltulosyl-glycine) and cysteine (Ota, Kohmura, & Kawaguchi, 2006). Thus the much decreased pH values of the Gly-Amadori/Cys reaction solution were mainly ascribed to the depletion of the amino group of cysteine to form certain relatively stable cyclic compounds. To gain insight into which cyclic compounds were formed, reaction solutions of the Gly-Amadori/Cys and Gly-Amadori alone systems were compared by HPLC-MS analysis (Fig. 3a-b). As shown in Fig. 3a, there is no peak of cysteine present, which should appear at 13.40 min if present, in the Gly-Amadori/Cys reaction solution. Instead, the peak of thiazolidine-4-carboxylic acid formed between the Gly-Amadori (1-deoxy-D-xylulos-1-yl-L-glycine) and cysteine (expressed as G-TTCA) was observed (Fig. 3a) and identified (Fig. 3c), suggesting a reaction between cysteine and the Gly-Amadori intermediates in the Gly-Amadori/Cys solution. More coincidentally, as shown in Fig. 4, according to the chromatographic peak areas (shown in the right y-axis) by HPLC-MS, the accumulation of G-TTCA reached its maximum value at 5 min, then quickly degraded to a very low level in 10 min, and even gradually decreased to 30 min. This change was very similar to that of the ratio of the absorbance at 294 nm over that at 420 nm (294 nm/420 nm) shown in the left y-axis of Fig. 4. Since the absorbance ratio of 294 nm/420 nm in the Maillard reaction solution is usually used to indicate the transformation extent of the colorless degradation products to the brown products (Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001), the coincidence of the parallel trend of the absorbance ratio in the Gly-Amadori/Cys solution to the accumulated amount of G-TTCA, suggested that the chemical transformation from the colorless degradation products to the brown products was dependent on the G-TTCA in the solution. With the degradation of the G-TTCA, the transformation extent was increased, which was reflected by the decrease of 294 nm/420 nm absorbance ratio (Fig. 4), and more browning products would be generated (Fig. 2b).

In Fig. 2, as for the three reaction model systems of Gly-Amadori/Cys, Cys-Amadori/Gly and TTCA/Gly, the Cys-Amadori/Gly system tended to give the highest reaction rate, followed by the Gly-Amadori/Cys system, and then the TTCA/Gly system, according to the observation of the 420 nm absorbance curve (Fig. 2a). In addition, the pH curve of the Gly-Amadori/Cys system was placed at the bottom while its 294 nm absorbance curve was in the highest place among the three reaction systems in the presence of a free amino acid (Fig. 2). As discussed above, this was because of the formation of G-TTCA in the Gly-Amadori/Cys reaction solution, where the occurrence of the lowest pH curve was a result of the depletion of the amino group of cysteine, and the highest 294 nm absorbance curve was a result of the delayed transformation from the colorless degradation products (294 nm) to the brown products (420 nm), as shown in Fig. 4. Otherwise, the

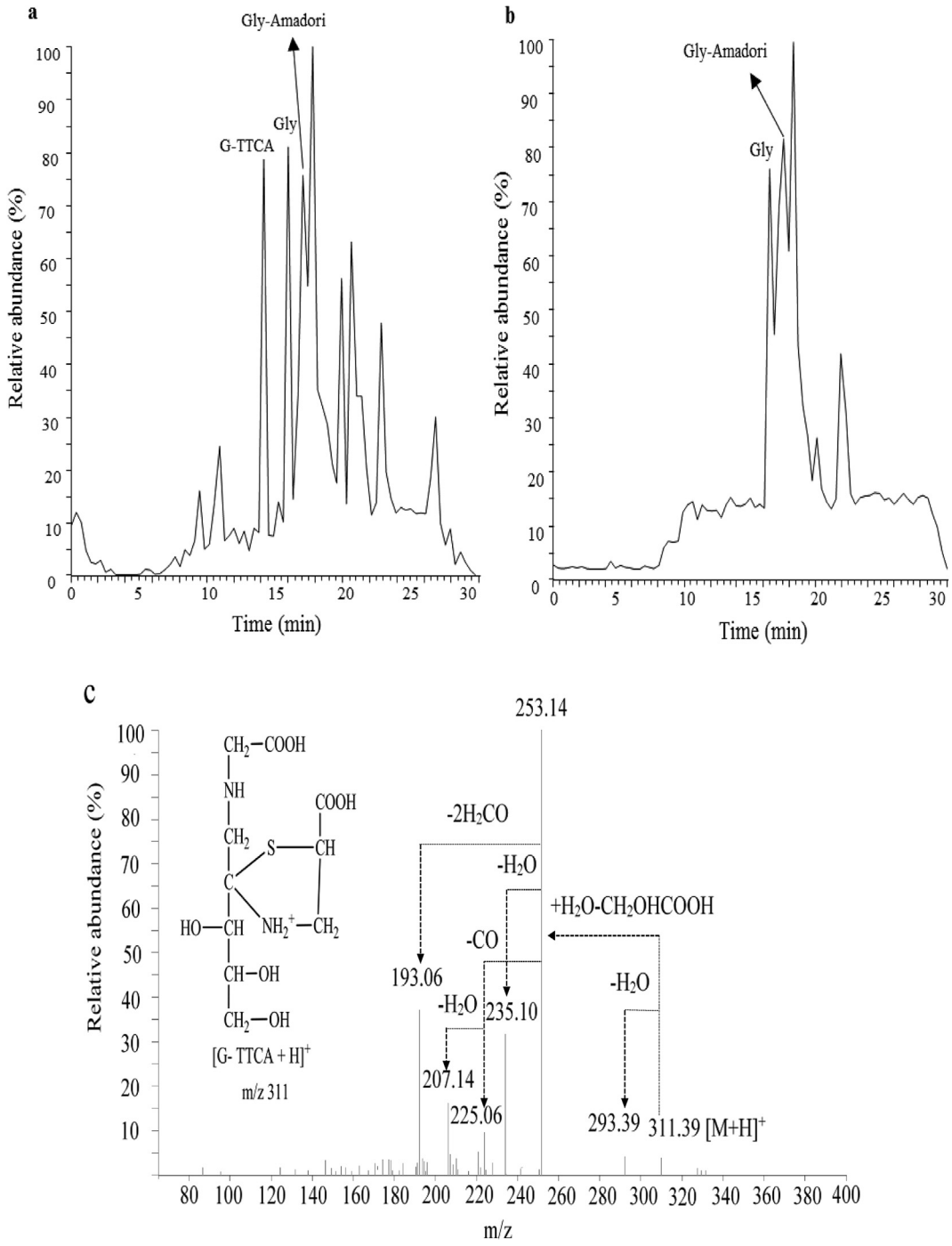

Fig. 3. Total ion current chromatograms in the analysis of the reaction solutions (a) Gly-Amadori/cysteine and (b) Gly-Amadori alone, which were sampled at a reaction time of 10 min, by HPLC-MS; (c) the corresponding MS/MS spectra of *m/z* 311 [M+H$^+$] for the G-TTCA peak (RT 14.19 min) marked in Fig. 3a of the Gly-Amadori/cysteine reaction solution. Note: tautomers or other possible isomers are not shown for the identified G-TTCA, i.e., a thiazolidine derivative formed between cysteine and Gly-Amadori.

formed G-TTCA should contribute no absorbance at 294 nm and 420 nm to the Gly-Amadori/Cys reaction solution, considering the TTCA, with similar chemical structure to the G-TTCA, has no absorbance at the two wavelengths. Noticeably, at the end of the reaction at 30 min (Fig. 2b), there was nearly no difference between the browning degrees (420 nm absorbance) of the Gly-Amadori/Cys solution and the Cys-Amadori/Gly solution, which was attributed to the aforementioned increasing degradation of G-TTCA in the Gly-Amadori/Cys solution and the higher reactivity of Gly-Amadori itself than the Cys-Amadori. Nevertheless, the TTCA/Gly system had consistent lower values of its browning degree than those of the Gly-Amadori/Cys or the Cys-Amadori/Gly system, in spite of the acceleration effect of glycine.

3.2. Volatile flavor compounds identified in the model systems

Sulfur-containing volatile compounds contribute significantly to meat aroma (Cerny & Davidek, 2003; Mottram, 1998). Under an acidic pH, mainly sulfur-containing compounds could be generated in a model reaction system containing cysteine and reducing sugars (Cerny & Davidek, 2003; Meynier & Mottram, 1995). In the present work, the mild technique of headspace solid-phase

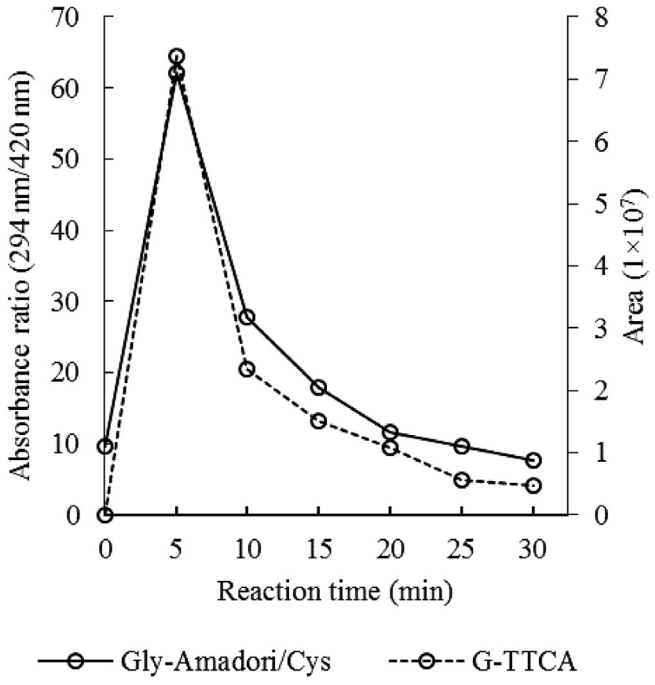

Fig. 4. For the reaction solution of Gly-Amadori/Cys, with reaction time, trend of 294 nm/420 nm UV/Vis absorbance ratio (shown in the *left* *y*-axis), in comparison with that of the peak area (shown in the *right* *y*-axis) of the accumulated G-TTCA by HPLC-MS analysis (see Fig. 3a). The data used in plotting the figures were means ± standard deviations (n = 3).

microextraction (SPME) was chosen to extract the volatile compounds in the reaction solutions, for sake of extraction of the thermally unstable sulfur-containing compounds (Hofmann, Schieberle, & Grosch, 1996). Besides, in addition to GC–MS analysis, GC-O was performed to find out which volatile compounds had odor activities that could contribute to overall aroma (Acree & Barnard, 1994). Due to their very low odor thresholds, in the GC-O of aroma extract dilution analysis (AEDA), usually it is time-consuming to get FD factors of the sulfur-containing compounds (Hofmann & Schieberle, 1995, 1997). In the present work, the AEDA/GC-O analysis was operated until the dilution ratio of 256. Based on the chemical identification by GC–MS analysis and the odors detected by GC-O analysis, the meat-like sulfur-containing compounds were characterized; in particular, those volatile compounds with FD values ≥256 were defined to be the potent aroma compounds.

The identified volatile compounds generated from the Gly-Amadori/Cys, TTCA/Gly, and Cys-Amadori/Gly systems are presented in Table 2. Two capillary columns including DB-5 and DB-Wax were used in the GC–MS analysis. However, only the DB-Wax column was used in the GC-O analysis, since most of the volatile compounds could be well separated and detected by this column. As shown in Table 2, a total of fifty-nine compounds were identified by the GC–MS, including forty-eight sulfur-containing compounds (thiols, sulfides, thiophenes, thiazoles, etc.), six nitrogen-containing heterocyclic compounds (pyridine, pyrazines, and pyrroles), and five oxygen-containing heterocyclic compounds (furans). As shown in Fig. S1, during the intermediate stage and the advanced stage of the Maillard reaction, the degradation of the Amadori compounds of Cys-Amadori or Gly-Amadori along with the Strecker degradation of cysteine and glycine, particularly, the reaction products of the reactive α-dicarbonyl compounds and H_2S, would be mainly involved in the formation of the aforementioned fifty-nine volatile compounds (Martins, Leussink, Rosing, Desclaux, & Boucon, 2010; Mottram, 1998; Mottram & Nobrega, 2002). Among them, fifty-five molecules were revealed to have odor-activities by GC-O. The high FD values (FD ≥ 64) were mainly attributed to the sulfur-containing compounds, while most of the identified nitrogen-containing or oxygen-containing heterocyclic compounds had small FD factors or had no odor-activities at all. In agreement with what had been reported on meat-like model reactions (Hofmann & Schieberle, 1995, 1997), the compounds generated from the three reaction systems listed in Table 2, including 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-furfurylthiol, 3-thiophenethiol, bis(2-methyl-3-furyl)disulfide, and 2-methylthiophene, most of which belonged to the thiol and thioether compounds, were found with powerful odor-activities (FD ≥ 256).

However, the total amount of sulfur-containing compounds of the three reaction systems had significant differences (see Table 2), among which Cys-Amadori/Gly had the highest amount, followed by TTCA/Gly, and then Gly-Amadori/Cys, according to the results obtained from both the HP-5 and the DB-Wax columns. This agreed with our previous investigation, aiming for the preparation technology of processed-meat flavorings, in the presence of an enzymatic hydrolyzate of meat (Gong et al., 2016). It was suggested that either the Gly-Amadori/Cys or the TTCA/Gly system would produce a lower amount of sulfur-containing compounds than the Cys-Amadori/Gly system, due to the aforementioned inhibitive effect of the relatively stable cyclic thiazolidine derivatives present in the two reaction systems. Noticeably, in comparison of the TTCA/Gly and Gly-Amadori/Cys systems, the former produced the higher total amount of sulfur-containing compounds, though, as discussed in 3.1, the latter showed a higher rate and degree of browning reaction than the former. The reason might be that the association of cysteine with Gly-Amadori in Gly-Amadori/Cys system reduced the Strecker degradation of cysteine to give H_2S, leading to fewer H_2S molecules being able to react with the colorless intermediate compounds (e.g., the α-dicarbonyl compounds) to give the sulfur-containing compounds (Mottram & Elmore, 2010; Wang & Ho, 2010) (see Fig. S1). On the other hand, glycine is apt to give browning reaction products in Maillard reaction, which was reflected by the 420 nm absorbance (Ashoor & Zent, 1984). As discussed in *Section* 3.1, because of the association of cysteine with Gly-Amadori, little free cysteine remained in the solution of Gly-Amadori/Cys (see Fig. 3a), which instead could give relatively more chances for the glycine that was split off from the Gly-Amadori to react with the colorless intermediate compounds, which were generated from the degradation of the Gly-Amadori (or the formed G-TTCA) and reflected by the 294 nm absorbance (see Fig. 2a), to produce the brown products (see Fig. 2b). However, in the TTCA/Gly system, the reversible conversion from TTCA to Cys-Amadori was accelerated by glycine (De Roos, 1992; De Roos et al., 2005; Gong et al., 2016). This acceleration would probably result in more Cys-Amadori degradation to yield H_2S, which could react with the colorless degradation compounds to produce the sulfur-containing compounds (Martins et al., 2010; Mottram, 1998) (see Fig. S1). Therefore, the reaction in the TTCA/Gly system was not as that in the Gly-Amadori/Cys system, where glycine was more favorable to react with the colorless intermediate compounds to produce the brown products (see Fig. 2b).

Besides, based on the total amount of the aforementioned six potent aroma compounds, including 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-furfurylthiol, bis(2-methyl-3-furyl) disulfide, 2-thiophenethiol, and 2-methylthiophene listed in Table 2, again it was found that the TTCA/Gly system produced significantly more meaty flavors than the Gly-Amadori/Cys system (*ca.* 5.0 *vs.* 2.3 in peak area), according to the results obtained from both the HP-5 and the DB-Wax columns. This meant potency of meaty flavor formed by the TTCA/Gly system was above two times greater than that formed by the Gly-Amadori/Cys system, in terms

Table 2
Results of GC–MS and GC-O analysis for the final reaction solutions of the Gly-Amadori/Cys, TTCA/Gly, and Cys-Amadori/Gly systems.

Compounds	[1]RI		[2]Peak areas($\times 10^5$)						[3]Odors	[3]FD factors			[4]Identification Methods
			DB-Wax			HP-5							
	DB-Wax	HP-5	a	b	c	a	b	c		a	b	c	
Sulfur-containing compounds													
Thiophene	1015	–	27.5^a	18.0^a	20.8^a	–	–	–	garlic	32	8	32	RI,MS,O,S
2-Methylthiophene	1093	782	443^a	441^a	337^b	312^a	323^a	258^b	sulfury	≥256	≥256	≥256	RI,MS,O,S
2,3-Dihydro-5-methylthiophene	1145	–	12.7 ^{b}	4.57^c	35.5^a	–	–	–	meaty	4	4	8	RI,MS,O
2,5-Dimethylthiophene	1156	–	23.4^b	9.05^c	32.3^a	–	–	–	meaty	32	16	32	RI,MS,O,S
2-Ethylthiophene	1170	–	3.92^b	–	7.11^a	–	–	–	meaty	32	16	64	RI,MS,O,S
2,3-Dimethylthiophene	1208	892	17.6^b	7.77^c	52.8^a	–	–	15.6	meaty	32	32	64	RI,MS,O,S
2-Methylthiazole	1234	–	9.79^b	16.1^a	18.0^a	–	–	–	cabbage	32	32	32	RI,MS,O,S
Thiazole	1244	736	19.2^c	29.5^a	25.2^b	15.1^c	26.5^a	20.9^b	meaty	16	32	64	RI,MS,O,S
3-Mercapto-2-butanone	1261	816	13.3^c	17.8^b	35.2^a	17.8^c	23.6^b	40.0^a	meaty	64	32	64	RI,MS,O,S
2-Ethylthiazole	1297	860	–	–	28.5	–	–	9.80	meaty	2	2	8	RI,MS,O,S
2-Methyl-3-furanthiol	1304	866	210^b	751^a	60.3^c	457^b	878^a	358^c	meaty	≥256	≥256	≥256	RI,MS,O,S
2,4-Dimethylthiazole	1309	917	–	–	139	–	–	105	meaty	4	8	64	RI,MS,O,S
4-Methylthiazole	1314	850	–	–	52.6	–	–	13.5	green, nutty	2	8	32	RI,MS,O,S
3-Mercapto-2-pentanone	1347	901	104^c	302^a	161^b	132^c	234^a	170^b	meaty	≥256	≥256	≥256	RI,MS,O,S
4,5-Dimethylthiazole	1361	–	–	–	11.3	–	–	–	meaty	32	32	64	RI,MS,O,S
2,4,5-Trimethylthiazole	1368	998	25.7^b	28.6^b	47.4^a	1.87^b	1.89^b	25.3^a	meaty	32	32	64	RI,MS,O,S
2-Ethyl-4-methylthiazole	1373	–	–	–	71.8	–	–	–	nutty	8	16	32	RI,MS,O,S
4-Ethyl-2,5-dimethylthiazole	1407	–	–	–	10.4	–	–	–	meaty	32	32	64	RI,MS,O
2-Furfurylthiol	1428	909	71.0^c	431^a	189^b	87.8^c	538^a	216^b	coffee	≥256	≥256	≥256	RI,MS,O,S
5-Ethyl-2,4-dimethylthiazole	1438	–	6.47^c	15.0^b	34.3^a	–	–	–	nutty	4	32	32	RI,MS,O
2-(2-Methylvinyl) thiophene	1480	–	–	–	27.3	–	–	–	sulfury	16	8	32	RI,MS,O
2,5-Diethyl-4-methylthiazole	1496	–	–	–	9.55	–	–	–	sulfury	8	8	32	RI,MS,O
Dihydro-2-methyl-3(2H)-thiophenone	1512	983	135^b	11.2^c	174^a	99.2^b	–	154^a	meaty	128	16	128	RI,MS,O,S
3-Thiophenethiol	1568	964	115^b	398^a	401^a	222^b	414^a	447^a	meaty	≥256	≥256	≥256	RI,MS,O,S
2-Acetylthiazole	1631	1015	28.0^b	23.8^b	39.4^a	14.87^b	13.50^b	42.3^a	popcorn	64	32	128	RI,MS,O,S
2-Thiophenecarboxaldehyde	1675	–	28.1^a	23.2^b	28.7^a	–	–	–	sulfury	8	16	16	RI,MS,O,S
2-Thiophenemethanethiol	1680	1050	–	–	–	62.53^c	188^a	156^b	meaty	64	128	128	RI,MS,O,S
2-Acetyl-3-methylthiophene	1750	1151	3.33	–	–	2.74	–	–	meaty	64	8	16	RI,MS,O,S
1,2,3-Trithiolane	1754	1104	13.7^b	3.01^c	28.0^a	1.95^b	2.19^b	51.0^a	sulfury	16	32	32	RI,MS,O
1-(3-Thienyl)-ethanone	1759	1044	3.57^b	2.14^c	6.09^a	–	–	–	garlic	2	4	8	RI,MS,O,S
5-Methyl-2-thiophenecarboxaldehyde	1766	1077	8.11^b	–	17.9^a	–	–	–	almond	64	16	64	RI,MS,O,S
2-Formyl-2,3-dihydrothiophene	–	1078	–	–	–	17.6^b	4.48^c	62.9^a	meaty	32	16	64	RI,MS,O
3-Methyl-2-thiophenecarboxaldehyde	1795	1115	102^b	61.2^c	123^a	91.0^b	45.5^c	103^a	almond	64	16	64	RI,MS,O,S
1-(2-Thienyl)-1-propanone	1826	1175	5.10^b	2.03^b	371 ^{a}	2.80^b	1.11^b	203^a	cream	4	4	64	RI,MS,O,S
Thieno[3,2-*b*]thiophene	1842	1201	154 ^{b}	102 ^{b}	247 ^{a}	65.7^b	60.2^b	238^a	metallic	16	8	32	RI,MS,O
2-Ethyl-5-propylthiophene	1893	–	–	–	64.8	–	–	–	garlic	4	16	32	RI,MS,O,S
2,5-Thiophenedicarboxaldehyde	1898	1235	32.2^b	38.4^b	43.5^a	25.2^b	21.56^b	47.7^a	sulfury	8	32	32	RI,MS,O,S
2-Methylthieno[2,3-*b*] thiophene	1932	1297	21.2^b	14.4^b	821^a	17.4^b	11.02^b	810^a	sulfury	16	16	64	RI,MS,O
Benzothiazole	1946	–	1.81	–	–	–	–	–	meaty	32	32	16	RI,MS,O,S
1-(2-Methyl-3-furylthio)-ethanethiol	–	1324	–	–	–	–	–	7.24	meaty	16	32	32	RI,MS,O
2-Methylthieno[3,2-*b*] thiophene	2032	1334	24.3^b	9.83^c	175^a	6.26^b	3.80^c	124^a	metallic	2	2	64	RI,MS,O
[1,2,3,4]Tetrathiane	–	1313	–	–	–	1.47^b	0.52^b	55.1^a	–	–	–	–	RI,MS
Bis(2-methyl-3-furyl)- disulfide	2156	1521	48.8^b	157^a	27.3^c	62.8^b	137^a	24.8^c	meaty	≥256	≥256	≥256	RI,MS,O,S
Bis(2-furfuryl)disulfide	2407	1631	2.38^b	10.5^a	2.75^b	–	5.13	–	meaty	16	16	16	RI,MS,O,S
3,3′-Dithiobisthiophene	2451	1845	5.31^b	14.2^a	6.55^b	1.92^b	6.93^a	2.35^b	meaty	1	2	1	RI,MS,O
2-Methyl-3- [(2-methyl-3-thienyl) dithio]furan	2453	1720	14.4^b	25.2^a	13.6^b	7.35^b	24.1^a	10.1^b	meaty	1	4	1	RI,MS,O
2,3-Dihydro-5-methyl-4-[(2-methyl-3-furyl)dithio]furan	2497	1676	20.7^b	51.9^a	14.5^c	30.7^b	52.7^a	22.4^c	burnt, meaty	32	64	64	RI,MS,O
2-([[(Thien-2-yl)thio] methyl] thio) thiophene	–	1897	–	–	–	2.68^b	5.30^a	5.88^a	–	–	–	–	RI,MS
Subtotal			1750^c	3020^b	4010^a	1760^c	3020^b	3800^a					
Nitrogen-containing heterocycles													
3-Methylpyridine	1231	–	3.90^b	3.17^b	8.79^a	–	–	–	nutty	8	8	16	RI,MS,O,S
2,5-Dimethylpyrazine	1314	–	–	–	28.6	–	–	–	roasted	2	8	16	RI,MS,O,S
2,3-Dimethylpyrazine	1335	–	3.67^b	–	30.5^a	–	–	–	roasted	4	1	8	RI,MS,O,S
Trimethylpyrazine	1396	–	–	–	9.09	–	–	–	burnt	4	4	64	RI,MS,O,S
Pyrrole	1508	–	2.43^c	3.14^b	4.18^a	–	–	–	burnt	4	8	16	RI,MS,O,S
1-(2-Furanylmethyl)-1H-pyrrole	1879	–	3.58	–	–	–	–	–	earthy	4	0	0	RI,MS,O
Subtotal			13.6 ^{b}	6.31 ^{c}	81.2 ^{a}	–	–	–					
Oxygen-containing heterocycles													
Furan	827	–	256^b	192^c	325^a	–	–	–	–	–	–	–	RI,MS
2-Methylfuran	888	621	436^b	305^c	533^a	230^b	192^c	256^a	–	–	–	–	RI,MS
Furfural	1456	833	282^a	61.3^b	76.2^b	240^a	60.9^a	75.2^b	caramel	≥256	32	32	RI,MS,O,S
2,2′-Bifuran	1585	–	70.5	–	–	–	–	–	caramel	8	4	4	RI,MS,O

Table 2 (*continued*)

Compounds	[1]RI		[2]Peak areas($\times 10^5$)						[3]Odors	[3]FD factors			[4]Identification Methods
			DB-Wax			HP-5							
	DB-Wax	HP-5	a	b	c	a	b	c		a	b	c	
2,2′-Methylenebisfuran	1602	–	49.1	–	–	–	–	–	caramel	128	4	16	RI,MS,O
Subtotal			1090[a]	558	934[b]	470[a]	253[c]	332[b]					
Total			2860[c]	3580[b]	5030[a]	2230[c]	3280[b]	4130[a]					

Note: "–", not detected.

[1] RI, linear retention indices determined using *n*-alkanes of C_6-C_{27} in the GC–MS analysis.

[2] a. Gly-Amadori/Cys; b. TTCA/Gly; c. Cys-Amadori/Gly. Results were the means of three replicates. Different subscript letters in the same row of the same column indicated significant differences ($p < 0.05$).

[3] The odor characteristics and FD values detected by the panelists in the GC-O analysis using a DB-Wax capillary column.

[4] RI, identified by retention indices (RI); MS, identified by comparison with mass spectra in the NIST 11 database and manual interpretation; O, identified by odor characteristics; S, identified by the comparison of the above analytical parameters with the standards injected.

of odor activity value (OAV). However, the Cys-Amadori/Gly system was observed to produce lower amounts for the six potent compounds than the TTCA/Gly system (Table 2). This was mainly because the Cys-Amadori/Gly system had generated, besides the predominant thiol and thioether compounds, more other sulfur-containing compounds with complex structures (e.g., 2,4-dimethylthiazole, and 2-methyl-thieno[3,2-*b*]thiophene) (Table 2), as a result of its high rate of reaction (see Fig. 2b).

Finally, the volatile compounds in the final reaction solutions of the Cys-Amadori alone, TTCA alone, and Gly-Amadori alone systems were also analyzed by GC–MS. The results are shown in Table S2. In general, the identified volatile compounds listed in Tables S2 and Table 2 were almost the same. However, the sulfur-containing compounds identified in Table S2 were observed to be fewer than those identified in Table 2. Furthermore, it was found either the TTCA alone or the Cys-Amadori alone system had generated a lower amount of sulfur-containing compounds than its counterpart of the TTCA/Gly or the Cys-Amadori/Gly system. This phenomenon suggested that the inclusion of glycine into the system of TTCA or Cys-Amadori could not only accelerate the browning reaction as discussed in *Section* 3.1, but also enhance the formation of meaty flavors. Notably, due to its slow rate of reaction in the absence of glycine (see Fig. 2), the TTCA alone system only could generate a low amount of sulfur-containing compounds (Table S2). Otherwise, as expected, no sulfur-containing flavors were found in the Gly-Amadori alone reaction system.

4. Conclusions

Regarding to the complex model reaction system of cysteine-xylose-glycine to generate meaty flavors, three possible intermediate products can be formed in the initial stage of the Maillard reaction. Among them, Gly-Amadori had the highest degradation rate, followed by the Cys-Amadori, and then the TTCA. Both of the pathways, Gly-Amadori with cysteine and TTCA with glycine, would encounter the resistant reactivity of a relatively stable thiazolidine derivative from cysteine. However, the former behaved more favorable to the formation of browning reaction products, while the latter was more favorable to the formation of sulfur-containing meaty flavors. Though the Cys-Amadori/glycine model system was found better than the TTCA/glycine model system in terms of the rate of reaction as well as the generated amount of meaty flavors, the pathway of TTCA with glycine is considered to be more feasible and practical for efficient preparation of processed-meat flavorings, since the intermediate product of Cys-Amadori is mainly converted from the TTCA in a reaction of cysteine-xylose-glycine. Considering the competitiveness between cysteine and glycine in the reaction with the reducing sugars, lower ratios of glycine used can favor the pathway of TTCA/Gly and increase the rate of reaction to form meaty flavors. However, excessive ratios of glycine used can help the generation of Gly-Amadori, which would benefit the formation of browning reaction products rather than the formation of meaty flavors. Therefore, to prepare processed-meat flavorings by thermal reactions composed of cysteine, reducing sugars, and glycine, it is of importance to optimize the ratio of each ingredient, particularly the glycine. In this way, the different kinds of initial intermediate products formed in the solution and the initial pathway, and also the rate of reaction would be regulated, which can meet the specific need on maximal formation of meaty flavors and the extent of browning reaction. However, with respect to a more complex reaction system to generate meaty flavors, such as real meat, more investigation is needed.

Acknowledgements

The present work was supported by National Natural Science Foundation of China (31371838, 31671895), and Beijing Municipal Natural Science Foundation (6172004).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.03.133.

References

Acree, T. E., & Barnard, J. (1994). Gas chromatography-olfactometry and CharmAnalysis. In H. Maarse & D. G. van der Heij (Eds.), *Trends in flavour research* (pp. 211–220). Amsterdam: Elsevier Science B.V.

Ajandouz, E. H., Tchiakpe, L. S., Dalle Ore, F., Benajiba, A., & Puigserver, A. (2001). Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science, 66*(7), 926–931.

Ashoor, S. H., & Zent, J. B. (1984). Maillard browning of common amino acids and sugars. *Journal of Food Science, 49*(4), 1206–1207.

Brands, C. M. J., & van Boekel, M. A. J. S. (2002). Kinetic modeling of reactions in heated monosaccharide-casein systems. *Journal of Agricultural and Food Chemistry, 50*(23), 6725–6739.

Cao, C. C., Liang, J. J., Wang, M., Hou, L., Xie, J. C., & Sun, B. G. (2016). Separation of amino acids and their initial Maillard reaction intermediates by hydrophilic interaction liquid chromatography. *Food Science (Shi Pin Ke Xue), 37*(2), 63–71 (In Chinese).

Cerny, C. (2007). Origin of carbons in sulfur-containing aroma compounds from the Maillard reaction of xylose, cysteine and thiamine. *LWT-Food Science and Technology, 40*(8), 1309–1315.

Cerny, C., & Davidek, T. (2003). Formation of aroma compounds from ribose and cysteine during the Maillard reaction. *Journal of Agricultural and Food Chemistry, 51*(9), 2714–2721.

Cerny, C., & Guntz-Dubini, R. (2013). Formation of cysteine-S-conjugates in the Maillard reaction of cysteine and xylose. *Food Chemistry, 141*(2), 1078–1086.

Chen, S.-L., Jin, S.-Y., & Chen, C.-S. (2005). Relative reactivities of glucose and galactose in browning and pyruvaldehyde formation in sugar/glycine model systems. *Food Chemistry, 92*(4), 597–605.

Davidek, T., Kraehenbuehl, K., Devaud, S., Robert, F., & Blank, I. (2005). Analysis of Amadori compounds by high-performance cation exchange chromatography coupled to tandem mass spectrometry. *Analytical Chemistry, 77*(1), 140–147.

de Roos, K. B. (1992). Meat flavor generation from cysteine and sugars. In R. Teranishi, G. R. Takeoka, & M. Guntert (Eds.), *Flavor precursors: Thermal and enzymatic conversions* (pp. 131–132). Washington DC: ACS Symposium Series; American Chemical Society.

de Roos, K. B., Wolswinkel, K., & Sipma, G. (2005). Amadori compounds of cysteine and their role in the development of meat flavor. In D. Weerasinghe & M. Sucan (Eds.), *Process and reaction flavors* (pp. 117–128). Washington DC: ACS Symposium Series; American Chemical Society.

Desclaux, G., Malik, T. I., Winkel, C., Pyle, D. L., & Mottram, D. S. (2006). Modelling the formation of Maillard reaction intermediates for the generation of flavour. In W. L. P. Bredie & M. A. Petersen (Eds.), *Flavour science: Recent advances and trends* (pp. 367–369). Amsterdam: Elsevier B.V..

Glomb, M. A., & Tschirnich, R. (2001). Detection of alpha-dicarbonyl compounds in Maillard reaction systems and in vivo. *Journal of Agricultural and Food Chemistry, 49*(11), 5543–5550.

Gong, J. X., Cao, C. C., Hou, L., Du, R. Q., Zhang, L., Xie, J. C., & Sun, B. G. (2016). The Initial-Maillard pathway to meat flavor during the preparation of thermal reaction meat flavorings. *Journal of Chinese Institute of Food Science and Technology (Zhong Guo Shi Pin Xue Bao), 16*(2), 68–75 (In Chinese).

Hao, Z., Lu, C.-Y., Xiao, B., Weng, N., Parker, B., Knapp, M., & Ho, C.-T. (2007). Separation of amino acids, peptides and corresponding Amadori compounds on a silica column at elevated temperature. *Journal of Chromatography A, 1147*(2), 165–171.

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry, 1*(15), 928–943.

Hofmann, T., & Schieberle, P. (1995). Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *Journal of Agricultural and Food Chemistry, 43*(8), 2187–2194.

Hofmann, T., & Schieberle, P. (1997). Identification of potent aroma compounds in thermally treated mixtures of glucose/cysteine and rhamnose/cysteine using aroma extract dilution techniques. *Journal of Agricultural and Food Chemistry, 45*(3), 898–906.

Hofmann, T., Schieberle, P., & Grosch, W. (1996). Model studies on the oxidative stability of odor-active thiols occurring in food flavors. *Journal of Agricultural and Food Chemistry, 44*(1), 251–255.

Huang, M. G., Zhang, X. M., Eric, K., Abbas, S., Hayat, K., Liu, P., ... Jia, C. S. (2012). Inhibiting the color formation by gradient temperature-elevating Maillard reaction of soybean peptide-xylose system based on interaction of L-cysteine and Amadori compounds. *Journal of Peptide Science, 18*(5), 342–349.

Jalbout, A. F., Shipar, M. A. H., & Navarro, J. L. (2007). Density functional computational studies on ribose and glycine Maillard reaction: Formation of the Amadori rearrangement products in aqueous solution. *Food Chemistry, 103*(3), 919–926.

Leiva, G. E., Naranjo, G. B., & Malec, L. S. (2017). A study of different indicators of Maillard reaction with whey proteins and different carbohydrates under adverse storage conditions. *Food Chemistry, 215*, 410–416.

Lerici, C. R., Barbanti, D., Manzano, M., & Cherubin, S. (1990). Early indicators of chemical changes in foods due to enzymic or nonenzymic browning reaction. 1: Study on heat-treated model systems. *Lebensmittel-Wissenschaft und-Technologie, 23*(4), 289–294.

Liu, S.-C., Yang, D.-J., Jin, S.-Y., Hsu, C.-H., & Chen, S.-L. (2008). Kinetics of color development, pH decreasing, and anti-oxidative activity reduction of Maillard reaction in galactose/glycine model systems. *Food Chemistry, 108*(2), 533–541.

Martins, S. I. F. S., Marcelis, A. T. M., & van Boekel, M. A. J. S. (2003). Kinetic modelling of Amadori N-(1-deoxy-d-fructos-1-yl)-glycine degradation pathways. Part I-Reaction mechanism. *Carbohydrate Research, 338*(16), 1651–1663.

Martins, S. I. F. S., Leussink, A., Rosing, E. A. E., Desclaux, G. A., & Boucon, C. (2010). Meat flavor generation in complex Maillard model systems: Reactants synergetic and/or competition effect? In D. S. Mottram & A. J. Taylor (Eds.), *Controlling maillard pathways to generate flavors* (pp. 71–83). Washington DC: ACS Symposium Series; American Chemical Society.

Meynier, A., & Mottram, D. S. (1995). The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chemistry, 52*(4), 361–366.

Mottram, D. S. (1998). Flavour formation in meat and meat products: a review. *Food Chemistry, 62*(4), 415–424.

Mottram, D. S., & Elmore, J. S. (2010). Control of the Maillard reaction during the cooking of food. In D. S. Mottram & A. J. Taylor (Eds.), *Controlling Maillard pathways to generate flavors* (pp. 143–155). Washington DC: ACS Symposium Series; American Chemical Society.

Mottram, D. S., & Nobrega, I. C. C. (2002). Formation of sulfur aroma compounds in reaction mixtures containing cysteine and three different forms of ribose. *Journal of Agricultural and Food Chemistry, 50*(14), 4080–4086.

Ota, M., Kohmura, M., & Kawaguchi, H. (2006). Characterization of a new Maillard type reaction product generated by heating 1-deoxymaltulosyl-glycine in the presence of cysteine. *Journal of Agricultural and Food Chemistry, 54*(14), 5127–5131.

Troise, A. D., Buonanno, M., Fiore, A., Monti, S. M., & Fogliano, V. (2016). Evolution of protein bound Maillard reaction end-products and free Amadori compounds in low lactose milk in presence of fructosamine oxidase I. *Food Chemistry, 212*, 722–729.

Wang, Y., & Ho, C.-T. (2010). Dicarbonyl intermediates: a control factor in the Maillard reaction. In D. S. Mottram & A. J. Taylor (Eds.), *Controlling Maillard pathways to generate flavors* (pp. 27–34). Washington DC: ACS Symposium Series; American Chemical Society.

Yang, Z., Xie, J. C., Zhang, L., Du, R. Q., Cao, C. C., Wang, M., ... Sun, B. G. (2015). Aromatic effect of fat and oxidized fat on a meat-like model reaction system of cysteine and glucose. *Flavour and Fragrance Journal, 30*(4), 320–329.

Yaylayan, V. A., & Huyghues-Despointes, A. (1994). Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Critical Reviews in Food Science and Nutrition, 34*(4), 321–369.

Yu, H., Seow, Y.-X., Ong, P. K. C., & Zhou, W. (2017). Effects of high-intensity ultrasound on Maillard reaction in a model system of D-xylose and L-lysine. *Ultrasonics Sonochemistry, 34*, 154–163.

Zeng, M.-M., Li, L.-L., He, Z.-Y., Qin, F., & Chen, J. (2012). Effect of glycine on Maillard reaction system and meat flavor compounds. *Food Science (Shi Pin Ke Xue), 33*(7), 32–36 (in Chinese).