Food Chemistry 345 (2021) 128761

Contents lists available at ScienceDirect

Food Chemistry

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

Reinvestigation of 2-acetylthiazole formation pathways in the Maillard reaction

Xiao-Yuan Wang [a], Yun-Jiao Ma [a], Yu Guo [a], Xiao-Lin Luo [a], Ming Du [a], Liang Dong [a], Pei Yu [b,*], Xian-Bing Xu [a,*]

[a] National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China
[b] College of Food Science and Engineering, Dalian Ocean University, Dalian 116036, China

ARTICLE INFO

Keywords:
2-Acetylthiazole
L-cysteine
D-glucose
Glyoxal
Formation pathway

ABSTRACT

2-Acetylthiazole possesses a nutty, cereal-like and popcorn-like aroma and a low odor threshold, and this compound has been identified in some processed foods, while the formation pathway of 2-acetylthiazole has not been clearly elucidated. Here, a model reaction of D-glucose and L-cysteine was constructed to investigate the formation pathway of 2-acetylthiazole. L-Cysteine, D-glucose and the corresponding intermediates, namely, dicarbonyl compounds (DCs), were involved in the formation of 2-acetylthiazole and detected by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS), high-performance ion chromatography (HPIC) and HPLC, respectively. The carbon module labeling (CAMOLA) technique revealed that the C-4 and C-5 of 2-acetylthiazole were derived from the carbons of glucose. The potential of glyoxal, which is degraded by glucose, to form 2-acetylthiazole was revealed for the first time. A novel route to form 2-acetylthiazole by the reaction of glyoxal and methylglyoxal produced by D-glucose with H_2S and NH_3 produced by L-cysteine was proposed.

1. Introduction

Flavor, one of the most important characteristics of food products, serves as an evaluation of consumer acceptance (Ames, Guy, & Kipping, 2001). The Maillard reaction is of particular importance for the formation of flavor compounds, such as heterocyclic flavor compounds (Mottram, 1994). Thiazoles, a class of heterocyclic flavor compounds, contain both nitrogen and sulfur atoms that are crucial for imparting a unique flavor to foods (Umano, Hagi, Nakahara, Shyoji, & Shibamoto, 1995). Thus, thiazoles frequently and substantially contribute to aroma, such as meaty flavor (Chen & Ho, 2002), cracker-like, and popcorn-like aromas (Buttery, Stern, & Ling, 1994; Pripis-Nicolau, de Revel, Bertrand, & Maujean, 2000) in processed foods. Thousands of thiazoles were also found in thermally processed food products such as popcorn (Chu & Yaylayan, 2009), meat (Mussinan & Walradt, 1974; Wilson, Mussinan, Katz, & Sanderson, 1973), potato (Yu, Zhou, & Yang, 2017), nut (Yu, Tan, & Shi, 2012), and milk (Arnold, Libbey, & Day, 2010; Scanlan, Lindsay, Libbey, & Day, 1968) products. In particular, thiazoles have been widely applied as flavor seasonings in imitation products meat to improve flavor quality (Wilson, 1975).

Over the years, the formation mechanisms of thiazoles, such as acyl thiazoles (Mulders, 1973; Sakaguchi & Shibamoto, 1978) and alkyl thiazoles (Sakaguchi & Shibamoto, 1978), have been investigated through the Maillard model reaction. In previous studies, the species of alkyl thiazoles identified in processed food are numerous, and alkyl thiazoles have been studied relatively extensively. Most acyl thiazoles have a sulfur flavor, whereas 2-acetylthiazole (Fig. 1) possesses a nutty, cereal-like flavor, projects a popcorn-like aroma with low odor thresholds (0.004–0.0041 mg/m^3), has a high odor intensity (Mottram, 1985; Pripis-Nicolau et al., 2000; Tao, Wu, Zhou, Gu, & Wu, 2014) and has been identified as an important compound in cooked red beans (Buttery, Seifert, & Ling, 1975), ground beef (Wilson et al., 1973), and pork liver (Mussinan & Walradt, 1974). According to previous reports, most researchers indicated that the C-4 and C-5 atoms of 2-acetylthiazole are derived from amino acids through the Maillard reaction of sugars and cysteine/cysteamine (Engel & Schieberle, 2002; Mulders, 1973; Sakaguchi & Shibamoto, 1978). A few researchers have shown that C-4 and C-5 come from dicarbonyl compounds (DCs) produced by sugars in a reaction involving DCs, hydrogen sulfide, and ammonia (Takken, 1976). However, the origins of C-4 and C-5 in 2-acetylthiazole have not been

* Corresponding authors. Tel.: +86 411 84763107; fax: +86 411 84763107 (P. Yu). Tel.: +86 411 86332275; fax: +86 411 86323262 (X.-B. Xu).
E-mail addresses: yupeiyuxiu@163.com (P. Yu), xianbingxu@gmail.com (X.-B. Xu).

https://doi.org/10.1016/j.foodchem.2020.128761
Received 17 August 2020; Received in revised form 15 October 2020; Accepted 27 November 2020
Available online 2 December 2020
0308-8146/© 2020 Elsevier Ltd. All rights reserved.

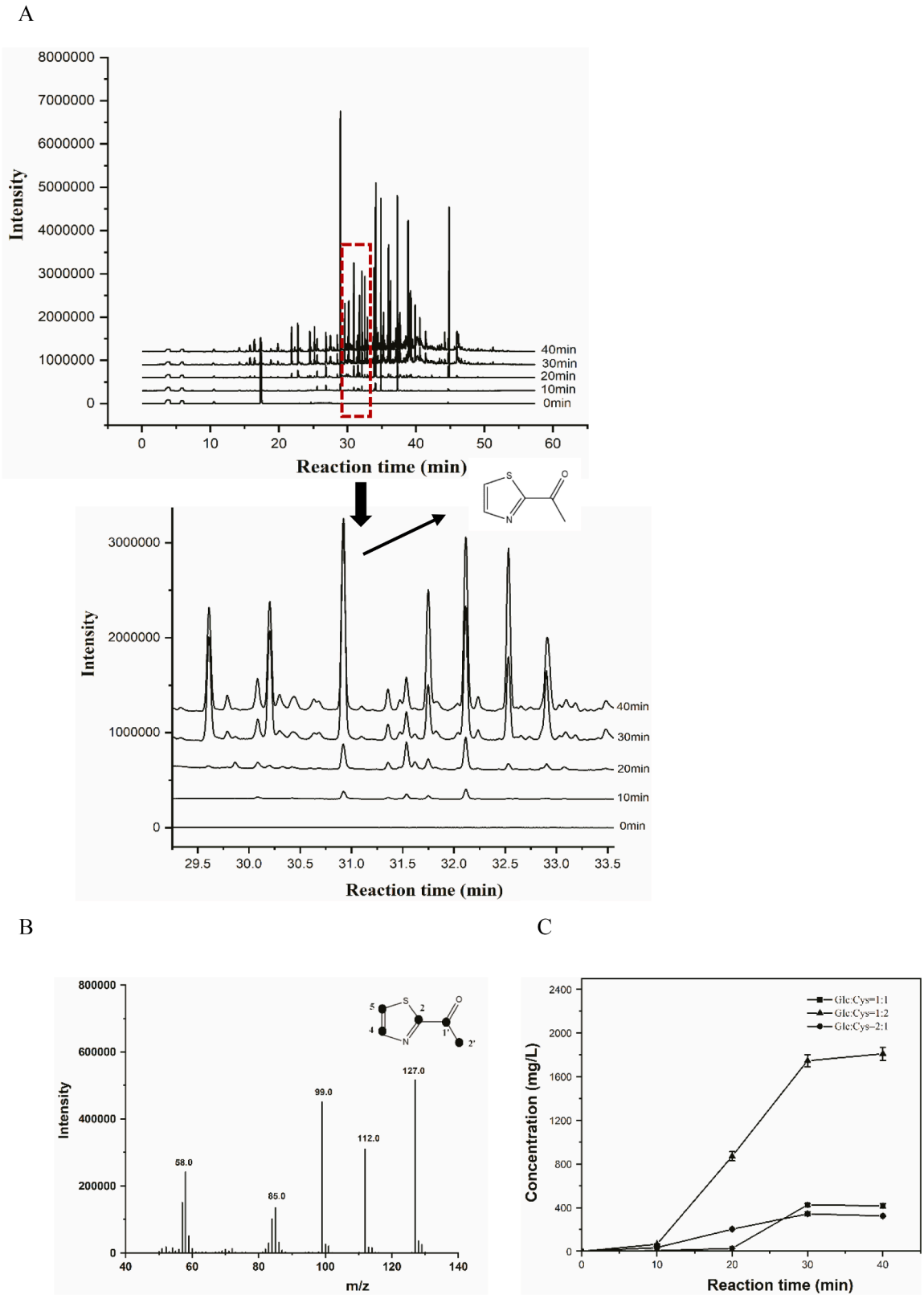

Fig. 1. The formation of 2-acetylthiazole with reaction time in the reaction of D-glucose and L-cysteine at three different ratios of 1:1, 1:2 and 2:1, and the resulting final concentrations of cysteine were 40, 40, and 80 mmol/L, respectively. (A) A chromatogram of 2-acetylthiazole and an enlarged graph. (B) A mass spectrum of 2-acetylthiazole. (C) The change in 2-acetylthiazole content with reaction time detected by GC-MS.

understood clearly, and some doubts have persisted as to why C-4- and C-5-substituted thiazolidines have not been detected if C-4 and C-5 are not derived from cysteine/cystine (Sakaguchi & Shibamoto, 1978; Takken, 1976). The hypothesis that C-4 and C-5 are derived from sugars has not been tested experimentally until now.

The aim of the present study was to examine whether C-4 and C-5 of 2-acetylthiazole are derived from the carbons of sugars. A Maillard model reaction between D-glucose and L-cysteine was performed to elucidate the reaction kinetics of precursors, intermediates, and 2-acetylthiazole. Additionally, the carbon module labeling (CAMOLA) technique was applied to trace the distribution of carbon atoms from glucose in the target compounds, and a verification experiment using ammonium sulfide instead of cysteine was conducted to evaluate the contribution of glyoxal to C-4 and C-5 in 2-acetylthiazole.

2. Materials and methods

2.1. Chemicals

D-Glucose (99.5%), L-cysteine (97%) and sodium hydroxide (chromatographic grade, 50%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). [$^{13}C_6$]-glucose (99%) was purchased from Cambridge Isotope Laboratories Inc. (Shanghai, China). Glyoxal (40%), methylglyoxal (40%), 3,4-hexanedione, formic acid (chromatographic grade), cyclohexanone, diethylene triamine pentacetic acid (DTPA, 99%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 99%) and o-phenylenediamine were all purchased from Aladdin (Shanghai, China). Methanol and acetonitrile were of chromatographic grade (99.9%) and obtained from Merck (Darmstadt, Germany). Ultrapure water was used throughout the experiments.

2.2. Maillard model reactions

A model system of a glucose-cysteine mixture in different molar ratios (the ratios of glucose to cysteine were 1:1, 1:2 and 2:1) was solubilized in ultrapure water. The resulting final concentrations of cysteine were 40, 40, and 80 mmol/L. The model reactions were carried out at 160 °C in high-pressure- and high-temperature-resistant glass bottles, and the reaction mixtures were sampled every 10 min. Subsequently, the mixtures were cooled immediately in an ice bath to terminate the reaction. Further analyses were carried out.

[$^{13}C_6$]-Glucose was reacted with L-cysteine at the same concentrations mentioned above at 160 °C for 30 min to trace the origin of carbon atoms. In a validation experiment, ammonium sulfide was used instead of L-cysteine in a series of reactions that were carried out at 160 °C for 30 min to further confirm that glyoxal was involved in the production of 2-acetylthiazole.

2.3. 2-Acetylthiazole analysis by headspace solid-phase microextraction combined with gas chromatography and mass spectrometry (HS-SPME-GC-MS)

The production of 2-acetylthiazole from the glucose-cysteine model system was analyzed by HS-SPME-GC-MS. Volatile compounds were extracted in a 10 mL headspace vial that was incubated in a water bath at 60 °C for 20 min. The SPME fiber (Divinylbenzene/Carboxen/Polydimethylsiloxane; 50/30 μm; Sigma-Aldrich, St. Louis, Missouri, USA) was then exposed to the headspace for 40 min in a headspace vial containing the sample. The extracted compounds were thermally desorbed by injecting the fiber in the injection port of the GC system at 250 °C for 5 min and operated in pulsed splitless mode.

The 7890B GC system (Agilent Technologies Inc., California, USA) was equipped with a 30 m × 250 μm × 0.25 μm VF-WAX-ms column. The carrier gas was helium with a flow rate of 1.7 mL/min and a column pressure of 125.489 kPa. The temperature of the injector and detector was 250 °C. The GC oven temperature program was set as follows: the oven was heated at 35 °C for 3 min, then the temperature was increased at a rate of 3 °C/min to 75 °C, which was maintained for 3 min, followed by increasing the temperature again at a rate of 5 °C/min to 240 °C, which was then maintained for 3 min. The GC column was directly connected to the ion source of an Agilent 5977B Series mass selective detector (California, USA) for mass spectrometric analysis.

2.4. Analysis of reducing sugars by high-performance ion chromatography (HPIC)

The reaction solution of D-glucose and L-cysteine was cleaned using C18 solid-phase extraction (SPE, Agela Technologies, Tianjin, China). Analysis of sugars was performed by using the Thermo Scientific™ Dionex™ ICS-5000$^+$ Capillary HPIC™ system (Thermo, Waltham, Massachusetts, USA), involving a pump (Dionex™ ICS-5000$^+$ DP), a column oven and a detector (Sekiguchi, Mitsuhashi, Inoue, Yagisawa, & Mimura, 2004) (Dionex™ ICS-5000$^+$ DC); 25 μL of clean solution was injected into a PA 1 column (Thermo, Waltham, Massachusetts, USA) with binary solvent gradient elution performed at 25 °C. Mobile phase A was water (100%), and a 250 mmol/L sodium hydroxide solution was used as mobile phase B with the following gradient program: 5% mobile phase B for 0–20 min, a linear gradient of 5% to 100% B from 20 to 26 min, followed by 100% B from 26 to 35 min; this was followed by 100% B to 5% from 35 to 36 min, and 5% B from 36 to 45 min at a flow rate of 0.25 mL/min (Feng et al., 2018).

2.5. Cysteine analysis by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS)

High-performance liquid chromatography (HPLC) instrument (Shimadzu LC-10AD binary pump, Kyoto, Japan) coupled with a triple quadrupole mass spectrometer (AB Sciex 4000 Qtrap System, Boston, USA) was employed to determine and quantify cysteine in the reaction solution. First, the reaction solution of D-glucose and L-cysteine was cleaned by C18 solid-phase extraction (SPE, Agela Technologies, Tianjin, China), and the eluate was collected for further analysis. Then, 10 μL of the cleaned solution was injected into a Phenomenex Synergi Fusion-RP C18 column (50 mm × 2 mm, 2.5 μm) (Los Angeles, California, USA) at 40 °C. The mobile phases consisted of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B), and isocratic elution was applied at 95% A at a flow rate of 0.3 mL/min.

Tandem mass spectrometry (MS/MS) was used to detect L-cysteine using multiple reaction monitoring (MRM) mode with positive ionization, and an L-cysteine standard was used to optimize the MRM parameters. The MRM parameters were as follows: declustering potential (DP), 40 V; collision energy (CE), 19 eV; and quantitative ion pair, 122/76 (m/z). The parameters of the electrospray ionization (ESI) source were set as follows: entrance potential (EP), 10 V; spray voltage, 5500 V; ion source temperature, 600 °C; and source gas and auxiliary gas, 15 and 18 L/min, respectively. High-purity nitrogen (99.9%) was used as the gas source.

2.6. Analysis of DCs by HPLC-diode array detector (DAD)

Eight microliters of internal standard (3,4-hexanedione, 50 g/L) and 2 mL of derivatization reagent obtained from o-phenylenediamine (2%) and solubilized in HEPES (1 mol/L, pH 7.0) at 11 mmol/L DTPA were added to the reaction solutions of D-glucose and L-cysteine (2 mL). The reaction mixtures were incubated under darkness for 2 h at room temperature and subsequently cleaned by C18-SPE (Feng et al., 2017; Xu, Yu, & Yu, 2017). Similarly, the reaction solution of glyoxal with ammonium sulfide and methylglyoxal with ammonium sulfide were also treated above.

The cleaned mixtures were analyzed by HPLC (Agilent Technologies Inc., California, USA) with an SPD-M20A DAD (Agilent Technologies Inc., California, USA) system. Ten microliters of cleaned mixture were

injected into a 5 μm SunFire TM 150 mm × 4.6 mm C18 column (Waters Co., Massachusetts, USA) for separation at 20 °C. The mobile phase consisted of water (100%, A) and methanol (100%, B). Gradient elution conditions of mobile phase B were set as follows: 5% at 0 min, then a linear gradient of 5% to 30% from 0 to 10 min, 30% to 100% from 10 to 45 min, and 100% methanol from 45 to 55 min at a flow rate of l mL/min. All o-phenylenediamine derivatives were detected with a diode array detector at a wavelength of 315 nm (Feng et al., 2017).

2.7. Statistical analysis

The data are presented as the mean and standard deviation. MultiQuant 2.1 was used to analyze MRM data on 4000 QTRAP. Agilent Mass Hunter Workstation Qualitative Analysis software B.08.00 was used for all GC-MS data processing.

3. Results and discussion

3.1. Analysis of 2-acetylthiazole

In the Maillard model reaction of D-glucose (40 mmol/L) and L-cysteine (40 mmol/L) (1:1), 2-acetylthiazole was detected (Fig. 1A) and identified by retention index (1640.4) and mass spectrometric data (m/z = 127) (Fig. 1B). As shown in Fig. 1A, an increase in 2-acetylthiazole was observed over time, but the contents of D-glucose and L-cysteine decreased gradually with reaction time, as observed upon measuring the precursors of the reaction (Fig. 2). The results indicated that D-glucose and L-cysteine may be involved in the production of 2-acetylthiazole in the model reaction (Marcus & Glomb, 2001).

To clarify the correlation between 2-acetylthiazole and the reaction precursors or intermediate degradation products, the effects of the concentrations of L-cysteine and D-glucose on the amount of 2-acetylthiazole were investigated further. When compared with the amount of 2-acetylthiazole measured with 40 mmol/L D-glucose (the ratio of D-glucose to L-cysteine is 1:1), slight changes were observed in the 2-acetylthiazole content when the concentration of D-glucose in the reaction system was increased to 80 mmol/L (the ratio of D-glucose to L-cysteine is 2:1) (Fig. 1C). However, the amount of 2-acetylthiazole significantly improved with an increase in L-cysteine (the ratio of D-glucose to L-cysteine was 1:2) (Fig. 1C). These results demonstrated that the concentration of L-cysteine in the reaction system highly affected the generation of 2-acetylthiazole. However, previous research has reported that 2-acetylthiazole was derived from L-cysteine as well as methylglyoxal obtained from glucose degradation (Mulders, 1973). This means that L-cysteine and D-glucose are equally important in the reaction system. Thus, L-cysteine, or its degradation products, might utilize other pathways to form 2-acetylthiazole.

3.2. Analysis of α-DCs

α-DCs, including glyoxal (retention time = 15.8 min) and methylglyoxal (retention time = 18.3 min), were isolated and identified by HPLC-DAD after o-phenylenediamine derivatization during the determination of thermal degradation products (Feng et al., 2017; Xu et al.,

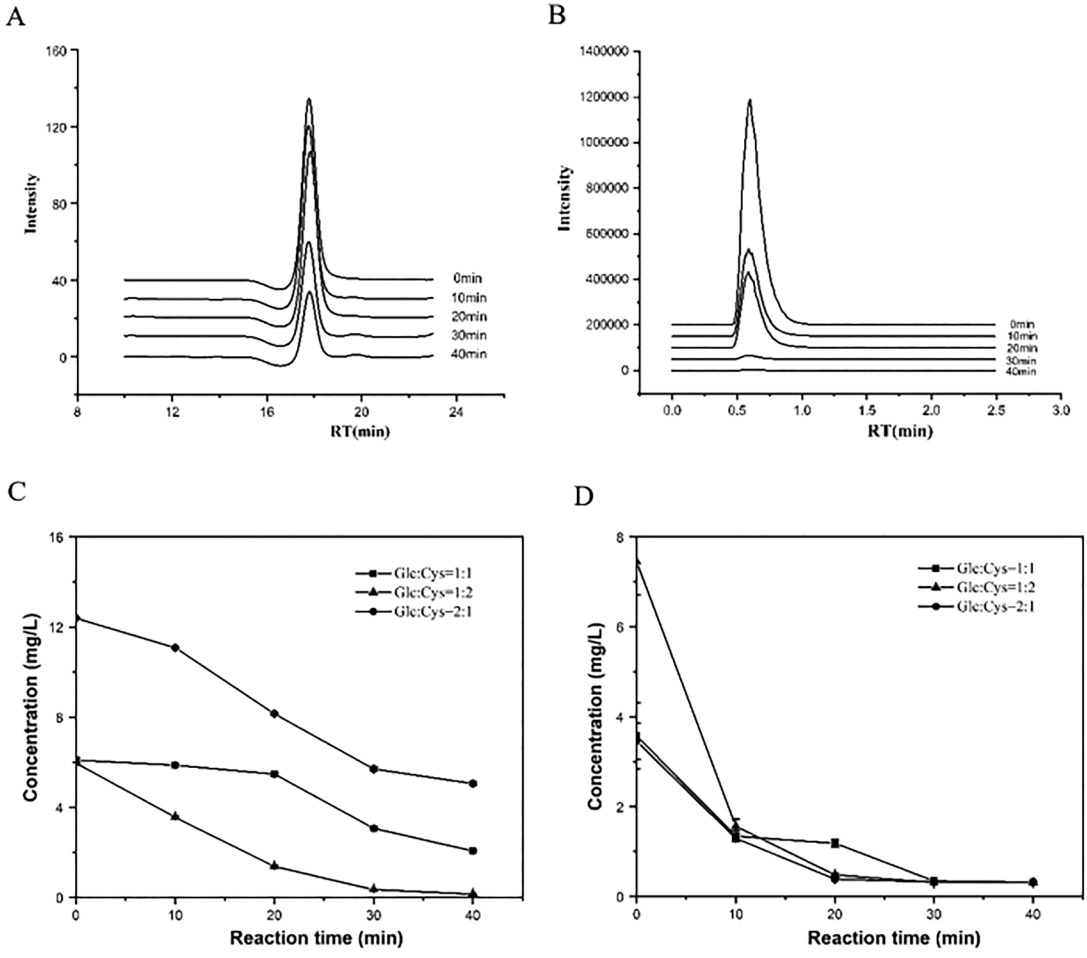

Fig. 2. The change in precursor contents with reaction time in the reaction of D-glucose and L-cysteine at three different ratios of 1:1, 1:2 and 2:1, and the resulting final concentrations of cysteine were 40, 40, and 80 mmol/L, respectively. (A) A chromatogram of D-glucose. (B) A chromatogram of L-cysteine. (C) The change in D-glucose content with reaction time. (D) The change in L-cysteine content with reaction time.

2017) that were obtained during the reaction and gradually increased with reaction time (Fig. 3). The results demonstrated that α-DCs, as an intermediate of the Maillard reaction (Hamzalıoğlu & Gökmen, 2016; Marcus & Glomb, 2001), also had a corresponding relationship with the formation of 2-acetylthiazole (Mulders, 1973).

As shown in Fig. 1C, when the ratio of D-glucose to L-cysteine was 1:2, the production rate of 2-acetylthiazole increased significantly between 10 and 20 min. Compared to the 1:1 ratio of glucose to cysteine, the consumption of D-glucose and L-cysteine was increased when the ratio of D-glucose to L-cysteine was 1:2 (Fig. 2C and D). In addition, with the increase in L-cysteine (the ratio of D-glucose to L-cysteine was 1:1 and 1:2), the production rate of glyoxal increased, while that of

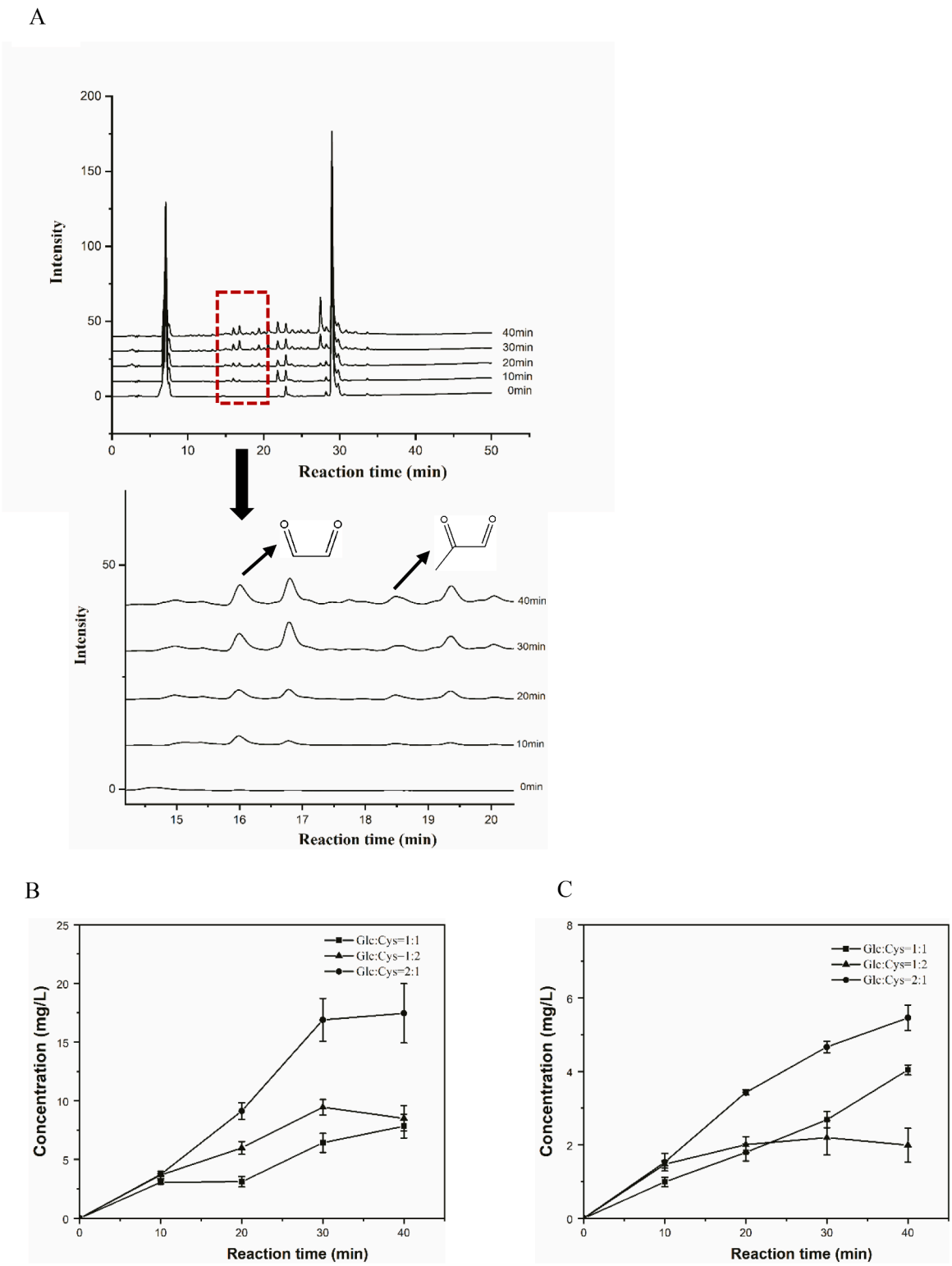

Fig. 3. The change in α-dicarbonyl compound (glyoxal, methylglyoxal) contents with reaction time in the reaction of D-glucose and L-cysteine at three different ratios of 1:1, 1:2 and 2:1, and the resulting final concentrations of cysteine were 40, 40, and 80 mmol/L, respectively. (A) A chromatogram of α-dicarbonyl compounds (glyoxal, methylglyoxal) and an enlarged graph. (B) The change in glyoxal content with reaction time. (C) The change in methylglyoxal content with reaction time.

methylglyoxal decreased (Fig. 3B and C). The results proved that 2-acetylthiazole was derived from L-cysteine and methylglyoxal obtained from glucose degradation (Mulders, 1973).

As shown in Fig. 2, the L-cysteine content was almost constant at three different ratios between 30 and 40 min, that of D-glucose was decreasing, the amount of 2-acetylthiazole only increased at the 1:2 ratio of glucose to cysteine (Fig. 1C), and the content of glyoxal and methylglyoxal decreased (Fig. 3B and C). A possible reason for this result was that glyoxal and methylglyoxal produced by D-glucose degradation reacted with the degradation products containing nitrogen and sulfur of L-cysteine to form 2-acetylthiazole in the reaction system. Therefore, L-cysteine may be involved in at least two reaction pathways during the formation of 2-acetylthiazole. As previously reported, L-cysteine was reacted with methylglyoxal to form 2-acetylthiazole (Mulders, 1973). Importantly, 2-acetylthiazole may also be formed by the degradation products of L-cysteine containing nitrogen and sulfur of L-cysteine and degradation products of glucose, such as glyoxal and methylglyoxal. However, a complex cascade reaction could be induced in the Maillard reaction of thermal degradation of carbohydrates and amines, in which the relationship between 2-acetylthiazole and α-DCs could be accurately determined (Yu & Ho, 2008). Therefore, the CAMOLA technique was performed further to determine the source of carbons in 2-acetylthiazole.

3.3. CAMOLA and validation experiments

To elucidate the relative contribution of D-glucose and L-cysteine to the formation of 2-acetylthiazole, [^{13}C6]-glucose was employed in the

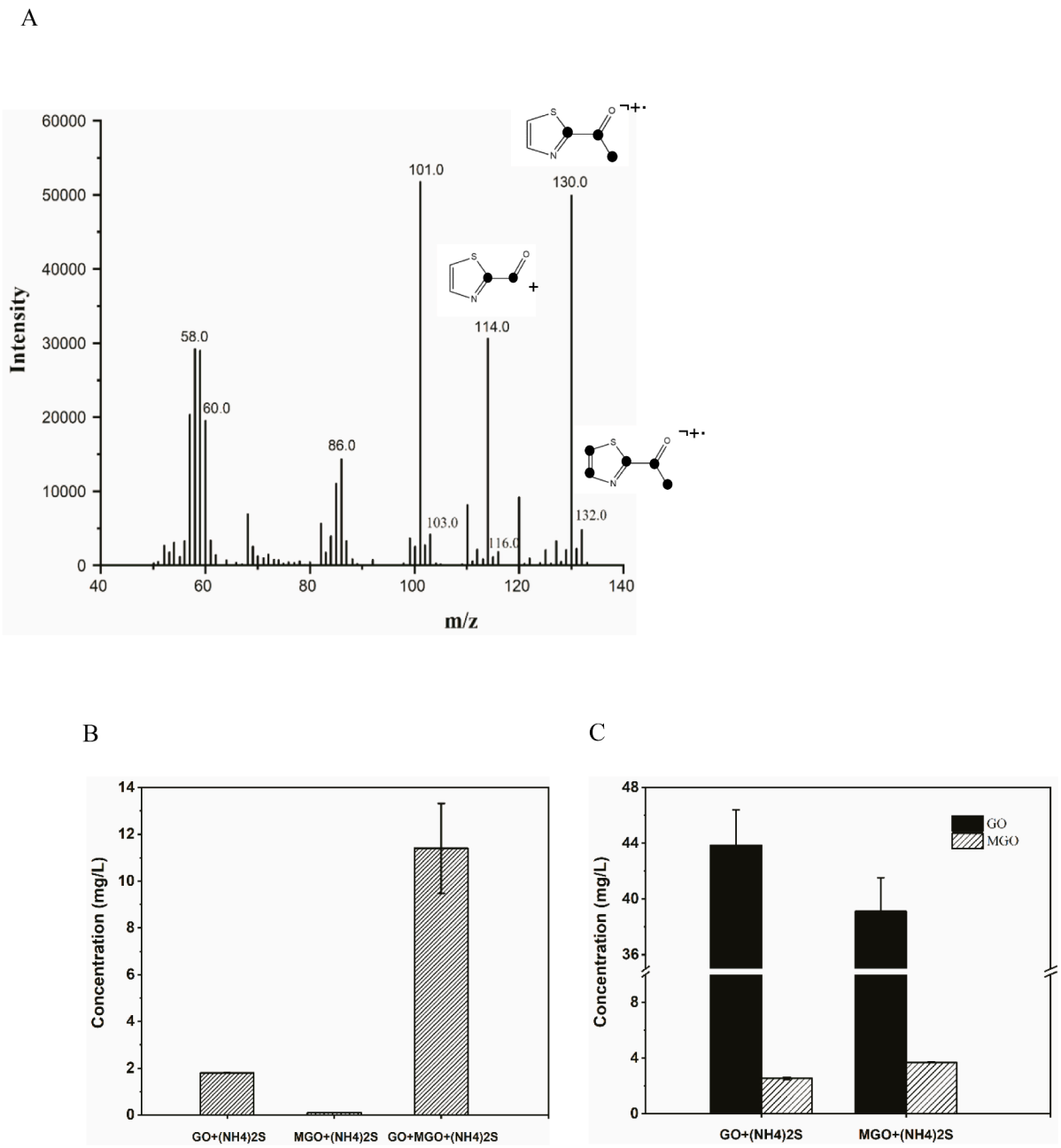

Fig. 4. (A) The mass spectrum of labeled 2-acetylthiazole (the mass-to-charge ratio of unlabeled 2-acetylthiazole is 127). (B) The content of 2-acetylthiazole detected by GC-MS in different reaction mixtures (glyoxal (GO) and $(NH_4)_2S$, methylglyoxal (MGO) and $(NH_4)_2S$, glyoxal (GO), methylglyoxal (MGO) and $(NH_4)_2S$). (C) The contents of glyoxal (GO) and methylglyoxal (MGO) detected by HPLC-DAD in different reaction mixtures (glyoxal (GO) and $(NH_4)_2S$, methylglyoxal (MGO) and $(NH_4)_2S$).

present study to trace the origin of the carbons in the 2-acetylthiazole structure. HS-SPME analysis in combination with GC-MS was used to analyze the 2-acetylthiazole produced from a mixture of [^{13}C6]-glucose and L-cysteine heated at 160 °C in three different proportions (the ratios of glucose to cysteine were 1:1, 1:2 and 2:1). The mass spectrum of labeled 2-acetylthiazole is shown in Fig. 4A. The mass peak of labeled 2-acetylthiazole [M + 3] ($m/z = 130$) was observed, and it accounted for the majority of the isotopic abundance ratio (82.89%) when [^{13}C6]-glucose reacted with L-cysteine at the 1:1 ratio of glucose to cysteine (Table 1). This result showed that the three carbon atoms in the 2-acetylthiazole originated from D-glucose. In addition, the loss of a methyl group in 2-acetylthiazole [M + 3] induced the formation of product ions ($m/z = 114$) with an abundance of 80.44% (Table 1). The results indicated that C-2, C-1′, and C-2′ of 2-acetylthiazole were derived from D-glucose degradation products such as methylglyoxal (Mulders, 1973).

Moreover, the [M + 5] peak of labeled 2-acetylthiazole ($m/z = 132$) was also obtained at a retention time of 30.9 min. The result suggested that 2-acetylthiazole contains five carbon atoms originating from glucose (Takken, 1976). Furthermore, the loss of a methyl group in 2-acetylthiazole [M + 5] ($m/z = 132$) induced the formation of product ions ($m/z = 116$) (Table 1), which showed that all four of the carbon atoms in the product ion also originated from glucose. In addition, the mass peak of [M + 5] ($m/z = 132$) and the product ions ($m/z = 116$) gradually increased with increasing glucose content (Table 1). Therefore, we proposed that C-4 and C-5 in 2-acetylthiazole might be derived from glucose degradation products with two carbons, such as glyoxal.

To evaluate the contribution of glyoxal to C-4 and C-5 in 2-acetylthiazole, a verification experiment using ammonium sulfide instead of cysteine was conducted. Cysteine could lead to the production of H_2S and NH_3 by Strecker degradation (Morra & Dick, 1991; Mottram, 1998; Smith, 1980), while H_2S and NH_3 are produced by the hydrolysis of ammonium sulfide (Kim, Lee, Kim, Kang, & Park, 2014). Thus, ammonium sulfide was used instead of cysteine in the reaction to further confirm that glyoxal was involved in the production of 2-acetylthiazole. 2-Acetylthiazole was detected when glyoxal reacted with methylglyoxal and ammonium sulfide (Fig. 4B). The results indicated that glyoxal was indeed involved in the production of 2-acetylthiazole. This means that C-4 and C-5 in 2-acetylthiazole could come from glyoxal, while C-2, C-1′, and C-2′ likely originate from methylglyoxal. Unexpectedly, 2-acetylthiazole was also observed in the reaction of glyoxal with ammonium sulfide and methylglyoxal with ammonium sulfide (Fig. 4B). In addition, methylglyoxal and glyoxal were detected in the reactions of glyoxal with ammonium sulfide and methylglyoxal with ammonium sulfide, respectively (Fig. 4C). This phenomenon implies that glyoxal and methylglyoxal can be converted to each other when ammonium sulfide is involved in the reaction, but a detailed mechanism should be investigated in future research.

3.4. The formation pathways of 2-acetylthiazole

Based on the CAMOLA approach, C-4 and C-5 in 2-acetylthiazole were mainly obtained from cysteine. A small portion of these atoms was also obtained from glucose. However, this contribution was insignificant. The formation pathways of 2-acetylthiazole from D-glucose and L-cysteine are shown in Fig. 5. The mechanism by which C-4 and C-5 are derived from cysteine/cysteamine has been extensively studied (Mulders, 1973; Sakaguchi & Shibamoto, 1978; Hofmann & Schieberle, 1995) (Fig. 5B). Importantly, the involvement of glyoxal in the formation of C-4 and C-5 in 2-acetylthiazole was proven, and a possible formation pathway from this method is shown in Fig. 5A. First, an attack of the thiol group on the carbonyl group of glyoxal is involved in the production of 2-hydroxymercaptoacetaldehyde (I) (Mottram, 1998). Ammonia produced by the degradation of L-cysteine reacts with I, thereby forming 2-hydroxymercaptoimine (II) (Zeng & Davies, 2006), which can be transformed into 2-mercapto vinylamine (III) by reduction and dehydration reactions. Furthermore, it can be assumed that the condensation of III and methylglyoxal produced by the degradation of D-glucose results in the formation of the aminoacetal IV, which can tautomerize into the intermediate V, enabling a nucleophilic attack of the thiol group with the formation of the thioacetal VI (Hofmann & Schieberle, 1995). Cyclization of VI, followed by β-elimination of water, yields 2-(l-hydroxyethyl)-thiazole (VI a). Therefore, it can be assumed that an oxidation step gives rise to 2-acetylthiazole (Engel & Schieberle, 2002; Kerler, van der Ven, & Weenen, 1997; Schieberle & Hofmann, 1996).

4. Conclusion

In summary, L-cysteine and D-glucose were thermally reacted to produce 2-acetylthiazole with a popcorn-like aroma, and the effects of different proportions of D-glucose and L-cysteine on 2-acetylthiazole were determined, demonstrating that the concentration of L-cysteine in the reaction system greatly affected the generation of 2-acetylthiazole. Importantly, based on the CAMOLA technique and the validation experiment, the C-4 and C-5 atoms in 2-acetylthiazole could also be derived from glucose, except for the carbon atoms derived from cysteine, and the involvement of glyoxal in the formation of C-4 and C-5 in 2-acetylthiazole was confirmed. Above all, the novel route of glyoxal and methylglyoxal produced by D-glucose with H_2S and NH_3 produced by L-cysteine was proposed to form 2-acetylthiazole. Unexpectedly, we also found that glyoxal and methylglyoxal can be converted to each other when ammonium sulfide is involved in the reaction, but a detailed mechanism should be investigated in future research.

CRediT authorship contribution statement

Xiao-Yuan Wang: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Yun-Jiao Ma:** Methodology, Validation, Formal analysis, Data curation. **Yu Guo:** Methodology, Validation, Formal analysis, Data curation. **Xiao-Lin Luo:** Methodology, Validation, Formal analysis. **Ming Du:** Methodology, Resources, Supervision. **Liang Dong:** Methodology, Data curation, Visualization. **Pei Yu:** Methodology, Resources, Visualization. **Xian-Bing Xu:** Conceptualization, Methodology, Resources, Investigation, Visualization, Funding acquisition.

Table 1
The isotopic abundance ratio of 2-acetylthiazole fragmentation ions with carbon labeling. [a]

	1:1[b]		1:2[b]		2:1[b]	
	$m/z = 127$	$m/z = 112$	$m/z = 127$	$m/z = 112$	$m/z = 127$	$m/z = 112$
M [c]	4.25 ± 0.07	5.84 ± 0.07	5.16 ± 0.03	5.79 ± 0.12	3.92 ± 1.55	10.30 ± 0.04
M + 2	3.52 ± 0.22	80.44 ± 0.01	3.43 ± 0.24	77.57 ± 0.23	3.51 ± 0.05	64.75 ± 0.38
M + 3	82.89 ± 0.01	2.85 ± 0.24	81.52 ± 0.23	2.87 ± 0.01	80.58 ± 1.25	3.14 ± 1.00
M + 4	2.64 ± 0.56	6.12 ± 0.17	3.11 ± 0.07	6.07 ± 0.01	3.12 ± 0.06	11.44 ± 0.09
M + 5	5.94 ± 0.29	–	5.88 ± 0.03	–	8.88 ± 0.3	–

[a] - not mentioned; values are shown by %mean±%RSD, n = 2.
[b] The three ratios (1:1, 1:2, 2:1) are the ratio of D-glucose to L-cysteine.
[c] M represents the relative molecular mass of 2-acetylthiazole (127).

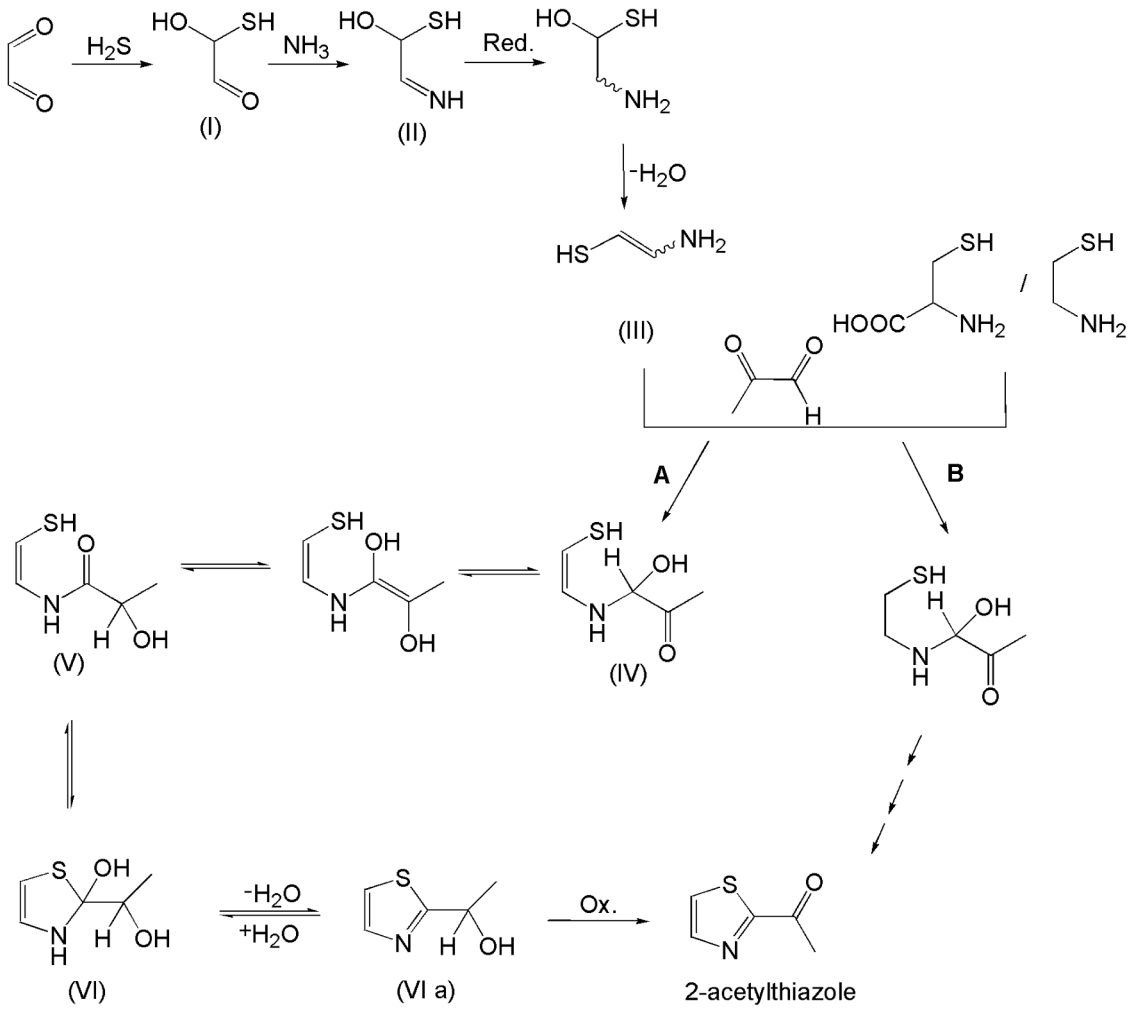

Fig. 5. The formation pathways of 2-acetylthiazole from D-glucose and L-cysteine. Pathway A is the mechanism by which C-4 and C-5 are derived from cysteine/cysteamine. Pathway B is the involvement of glyoxal in the formation of C-4 and C-5 in 2-acetylthiazole.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the 13th Five Years Key Programs for Science and Technology Department of China (Grant No. 2017YFD0400103), the Superior College Science Technology Research Project of Liaoning Province of China (Grant NO. QL201709) and Central Funds Guiding the Local Science and Technology Development of China (2020JH6/10500002).

References

Ames, J. M., Guy, R. C. E., & Kipping, G. J. (2001). Effect of pH, temperature, and moisture on the formation of volatile compounds in glycine/glucose model systems. *Journal of Agriculture and Food Chemistry, 49*(9), 4315–4323. https://doi.org/10.1021/jf010198m.

Arnold, R. G., Libbey, L. M., & Day, E. A. (2010). Identification of components in the stale flavor fraction of sterilized concentrated milk. *Journal of Food Ence, 31*(4), 566–573.

Buttery, R. G., Seifert, R. M., & Ling, L. C. (1975). Characterization of some volatile constituents of dry red beans. *Journal of Agriculture and Food Chemistry, 23*(3), 516–519. https://doi.org/10.1021/jf60199a053.

Buttery, R. G., Stern, D. J., & Ling, L. C. (1994). Studies on flavor volatiles of some sweet corn products. *Journal of Agriculture and Food Chemistry, 42*(3), 791–795. https://doi.org/10.1021/jf00039a038.

Chen, Y., & Ho, C.-T. (2002). Effects of carnosine on volatile generation from Maillard reaction of ribose and cysteine. *Journal of Agriculture and Food Chemistry, 50*(8), 2372–2376. https://doi.org/10.1021/jf011244l.

Chu, F. L., & Yaylayan, V. A. (2009). Isotope labeling studies on the origin of 3,4-hexanedione and 1,2-butanedione in an alanine/glucose model system. *Journal of Agriculture and Food Chemistry, 57*(20), 9740–9746. https://doi.org/10.1021/jf902117v.

Engel, W., & Schieberle, P. (2002). Identification and quantitation of key aroma compounds formed in Maillard-type reactions of fructose with cysteamine or isothiaproline (1,3-thiazolidine-2-carboxylic acid). *Journal of Agriculture and Food Chemistry, 50*(19), 5394–5399. https://doi.org/10.1021/jf0203186.

Feng, T.-T., Wu, J.-H., Liang, X.u., Du, M., Tan, M.-Q., Qin, L., & Xu, X.-B. (2018). Quality properties and formation of α-dicarbonyl compounds in abalone muscle (*Haliotis discus*) as affected by tenderization and baking processes. *Food Measure, 12*(3), 1503–1512. https://doi.org/10.1007/s11694-018-9765-9.

Feng, T.-T., Xu, X.-B., Du, M., Tan, M.-Q., Qin, L., & Zhu, B.-W. (2017). Simultaneous determination of glyoxal, methylglyoxal and diacetyl in beverages using vortex-assisted liquid–liquid microextraction coupled with HPLC-DAD. *Analytical Methods, 9*(16), 2445–2451. https://doi.org/10.1039/C7AY00180K.

Hamzalıoğlu, A., & Gökmen, V. (2016). Investigations on the reactions of α-dicarbonyl compounds with amino acids and proteins during in vitro digestion of biscuits. *Food & Function, 7*(6), 2544–2550. https://doi.org/10.1039/C5FO01555C.

Kerler, J., van der Ven, J. G. M., & Weenen, H. (1997). α-Acetyl- N -heterocycles in the Maillard reaction. *Food Reviews International, 13*(4), 553–575. https://doi.org/10.1080/87559129709541140.

Kim, M.-I., Lee, G.-H., Kim, D.-W., Kang, D.-H., & Park, D.-W. (2014). Production of elemental sulfur and ammonium thiosulfate by H_2S oxidation over Nb_2O_5 supported on Fe-pillared clay. *Korean Journal of Chemical Engineering, 31*(12), 2162–2169. https://doi.org/10.1007/s11814-014-0167-3.

Marcus, A., Glomb, Roswitha, & Tschirnich. (2001). Detection of α-Dicarbonyl Compounds in Maillard Reaction Systems and in Vivo. Journal of Agricultural and Food Chemistry, 49(11), 5543–5550.

Morra, M. J. & Dick, W. A. (1991). Mechanisms of H2S production from cysteine and cystine by microorganisms isolated from soil by selective enrichment. Applied & Environmental Microbiology, 57(5), 1413–1417.

Mottram, Donald S. (1985). The effect of cooking conditions on the formation of volatile heterocyclic compounds in pork. *Journal of the Science of Food and Agriculture, 36*(5), 377–382. https://doi.org/10.1002/jsfa.2740360510.

Mottram, D. S. (1994). Flavor compounds formed during the Maillard reaction. *ACS Symposium Series, 543*, 104–126.

Mottram, Donald S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415–424. https://doi.org/10.1016/S0308-8146(98)00076-4.

Mulders, E. J. (1973). Volatile components from the non-enzymic browning reaction of the cysteine/cystine-ribose system. Zeitschrift Fuer Lebensmittel Untersuchung Und Forschung, 152(4), 193-201.

Mussinan, Cynthia J., & Walradt, John P. (1974). Volatile constituents of pressure cooked pork liver. *Journal of Agriculture and Food Chemistry, 22*(5), 827–831. https://doi.org/10.1021/jf60195a002.

Pripis-Nicolau, Laura, de Revel, Gilles, Bertrand, Alain, & Maujean, Alain (2000). Formation of Flavor Components by the Reaction of Amino Acid and Carbonyl Compounds in Mild Conditions. *Journal of Agriculture and Food Chemistry, 48*(9), 3761–3766. https://doi.org/10.1021/jf991024w.

Sakaguchi, Minoru, & Shibamoto, Takayuki (1978). Formation of heterocyclic compounds from the reaction of cysteamine and D-glucose, acetaldehyde, or glyoxal. *Journal of Agriculture and Food Chemistry, 26*(5), 1179–1183. https://doi.org/10.1021/jf60219a056.

Scanlan, R. A., Lindsay, R. C., Libbey, L. M., & Day, E. A. (1968). Heat-Induced Volatile Compounds in Milk. *Journal of Dairy Science, 51*(7), 1001–1007. https://doi.org/10.3168/jds.S0022-0302(68)87113-9.

Schieberle, P., & Hofmann, T. (1996). Identification of the Key Odorants in Processed Ribose-Cysteine Maillard Mixtures by Instrumental Analysis and Sensory Studies. Flavour Science, 15(2), 175–181.

Smith, H. I. K. (1980). Cysteine Metabolism in Cultured Tobacco Cells. Plant Physiology, 65(1), 151–155.

Takken. (1976). Reaction Products of α-Dicarbonyl Compounds, Aldehydes, Hydrogen Sulfide, and Ammonia. *ACS Symposium Series, 26*(7), 114–121.

Tao, Ning-Ping, Wu, Rong, Zhou, Pei-Gen, Gu, Sai-Qi, & Wu, Wei (2014). Characterization of odor-active compounds in cooked meat of farmed obscure puffer (Takifugu obscurus) using gas chromatography–mass spectrometry–olfactometry. *Journal of Food and Drug Analysis, 22*(4), 431–438. https://doi.org/10.1016/j.jfda.2014.02.005.

Hofmann, Thomas, & Schieberle, Peter (1995). Studies on the Formation and Stability of the Roast-Flavor Compound 2-Acetyl-2-thiazoline. *Journal of Agriculture and Food Chemistry, 43*(11), 2946–2950. https://doi.org/10.1021/jf00059a032.

Umano, Katsumi, Hagi, Yukio, Nakahara, Kazuaki, Shyoji, Akihiro, & Shibamoto, Takayuki (1995). Volatile Chemicals Formed in the Headspace of a Heated D-Glucose/L-Cysteine Maillard Model System. *Journal of Agriculture and Food Chemistry, 43*(8), 2212–2218. https://doi.org/10.1021/jf00056a046.

Wilson, Richard A. (1975). Review of thermally produced imitation meat flavors. *Journal of Agriculture and Food Chemistry, 23*(6), 1032–1037. https://doi.org/10.1021/jf60202a016.

Wilson, Richard A., Mussinan, Cynthia J., Katz, Ira, & Sanderson, Anne (1973). Isolation and identification of some sulfur chemicals present in pressure-cooked beef. *Journal of Agriculture and Food Chemistry, 21*(5), 873–876. https://doi.org/10.1021/jf60189a028.

Xu, Xian-Bing, Yu, Pei, & Yu, Shu-Juan (2017). Promotion effect of sulfite on deoxyosones and 4-methylimidazole in caramel model system. *Food Chemistry, 223*, 121–127. https://doi.org/10.1016/j.foodchem.2016.12.033.

Sekiguchi, Yoko, Mitsuhashi, Naoto, Inoue, Yoshinori, Yagisawa, Hitoshi, & Mimura, Tetsuro (2004). Analysis of sugar phosphates in plants by ion chromatography on a titanium dioxide column with pulsed amperometric detection. *Journal of Chromatography A, 1039*(1-2), 71–76. https://doi.org/10.1016/j.chroma.2004.02.015.

Yu, Ai-Nong, Tan, Zhi-Wei, & Shi, Bo-An (2012). Influence of the pH on the formation of pyrazine compounds by the Maillard reaction of L-ascorbic acid with acidic, basic and neutral amino acids. *Asia-Pacific Journal of Chemical Engineering, 7*(3), 455–462. https://doi.org/10.1002/apj.594.

Yu, Ai-Nong, Zhou, Yong-Yan, & Yang, Yi-Ni (2017). Kinetics of browning and correlations between browning degree and pyrazine compounds in l-ascorbic acid/acidic amino acid model systems. *Food Chemistry, 221*, 1678–1684. https://doi.org/10.1016/j.foodchem.2016.10.119.

Yu, Wang, & Ho, Chi-Tang (2008). Formation of 2,5-Dimethyl-4-hydroxy-3(2 H)-furanone through Methylglyoxal: A Maillard Reaction Intermediate. *Journal of Agriculture and Food Chemistry, 56*(16), 7405–7409. https://doi.org/10.1021/jf8012025.

Zeng, Jingmin, & Davies, Michael J. (2006). Protein and Low Molecular Mass Thiols as Targets and Inhibitors of Glycation Reactions. *Chemical Research in Toxicology, 19*(12), 1668–1676. https://doi.org/10.1021/tx0602158.