



Effect of glycine on reaction of cysteine-xylose: Insights on initial Maillard stage intermediates to develop meat flavor



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ABSTRACT

The meat-like reactions of L-cysteine and D-(+)-xylose with or without glycine were investigated. LC-MS was used to quantitatively determine the initial stage intermediates including 2-threityl-thiazolidine-4-carboxylic acid, Cys-Amadori, and Gly-Amadori in the reaction mixtures. The results showed that the addition of glycine was only in positive correlation with the browning feature of cysteine-xylose reaction. When excessive glycine was added, a high browning rate would be achieved, but it did not benefit the formation of meaty compounds. For a complex meat-like reaction system containing cysteine, reducing sugars and glycine, to overcome the low rate of reaction, and particularly, to minimize the inhibitive effect of cysteine in the generation of meaty flavors, selection of an appropriate ratio between cysteine and glycine is important in an effort to make amounts of the intermediates of Cys-Amadori and Gly-Amadori approximately equally consist in the reaction mixture.

1. Introduction

The Maillard reaction, also called non-enzymatic browning reaction, involves many different pathways, leading to production of a wide range of chemicals, which include a large number of volatile compounds found in cooked foods (Leiva, Naranjo, & Malec, 2017; Mottram & Elmore, 2010). According to the Hodge-scheme (Hodge, 1953), there are three reaction stages to go through in the Maillard reaction, namely, the initial (early) stage, the intermediate stage, and the advanced (final) stage. The Amadori rearrangement takes place in the initial stage between aldoses and amino compounds such as amino acids, which forms Amadori rearrangement products (ARPs). ARPs are the primary reaction intermediates that can be decomposed into α -dicarbonyls, which are the very reactive intermediate products that can initiate a cascade of further reactions, resulting in the formation of a complex mixture containing volatile flavor compounds and browning pigments, of which the latter also are referred to as melanoidins (Glomb & Tschirnich, 2001; Jalbout, Shipar, & Navarro, 2007; Troise, Buonanno, Fiore, Monti, & Fogliano, 2016; Wang & Ho, 2010).

Cysteine is an important precursor of sulfur-containing compounds for the development of meat flavor (Cerny & Guntz-Dubini, 2013). As shown in Fig. 1, in a meat-like Maillard reaction system composed of cysteine and reducing sugars, such as xylose, in the initial Maillard

reaction stage, it is the relatively stable cyclic 2-threityl-thiazolidine-4-carboxylic acids (TTCA) that are predominately formed (de Roos, 1992; de Roos, Wolswinkel, & Sipma, 2005; Gong et al., 2016), which leads to the cascade of reactions from cysteine to develop meaty flavors being circumvented. Besides, the formation of the relatively stable thiazolidines induced by cysteine can also result in inhibition of browning in Maillard reaction (Huang et al., 2011; Molnar-Perl & Friedman, 1990; Seo & Karboune, 2014). However, it is interesting that, under heating, TTCA can be reversibly converted to the Amadori rearrangement product of cysteine (Cys-Amadori), and the conversion can be acid-catalyzed by other amino acids (de Roos, 1992; Gong et al., 2016).

Glycine is the simplest amino acid present in food, and is usually applied in the preparation of processed-meat flavorings (Wong, Aziz, & Mohamed, 2008). It was reported that the reaction between cysteine and reducing sugars to form meat-like flavors can be accelerated by glycine (de Roos, 1992; Gong et al., 2016; Martins, Leussink, Rosing, Desclaux, & Boucon, 2010). However, for the complex reaction system composed of cysteine, reducing sugar, and glycine, glycine and cysteine can competitively react with the reducing sugar, resulting in the formation of three initial stage intermediate products as shown for xylose in Fig. 1, i.e., TTCA, Cys-Amadori and Gly-Amadori (the Amadori rearrangement product of glycine). Therefore, the sulfur-containing meaty flavors can be developed either via the degradation of

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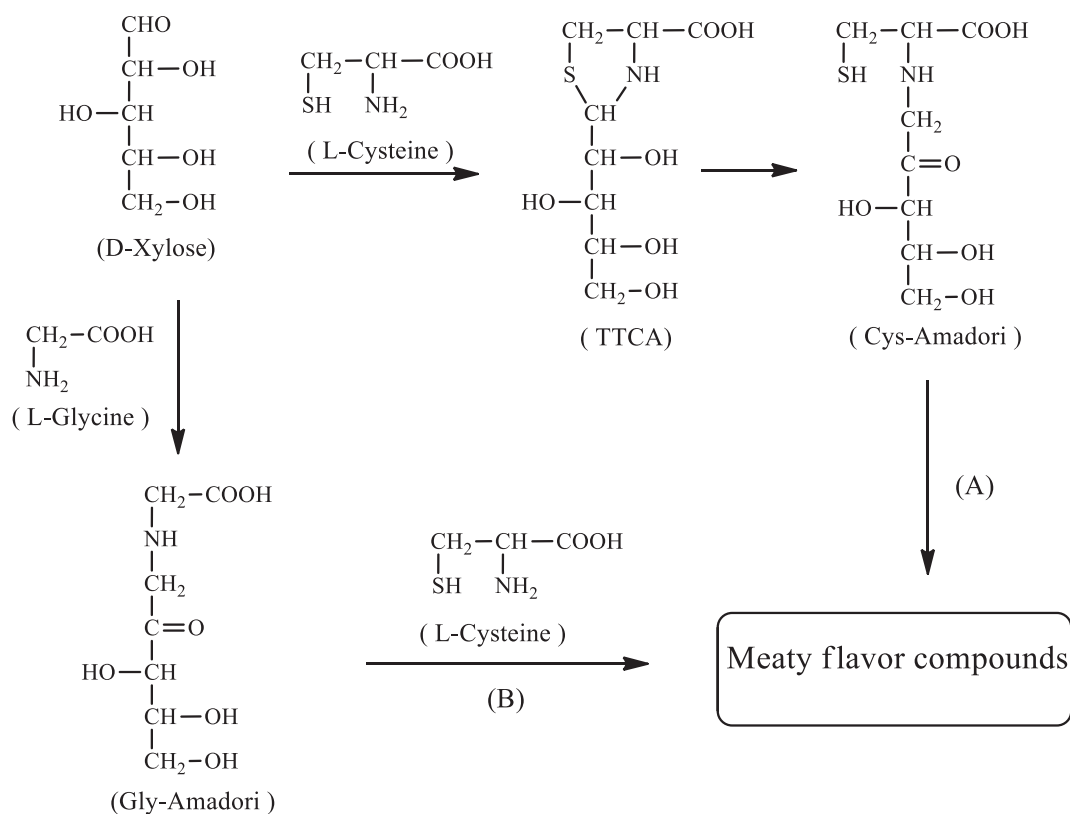


Fig. 1. According to the initial Maillard reaction stage, the two major pathways to develop meaty flavors in reaction systems of cysteine-xylose-glycine. Note. TTCA, 2-threitylthiazolidine-4-carboxylic acid. There are tautomers, cyclic structures, or ionic structures for the Amadori compounds and the TTCA, while only their open-chain forms are shown in this figure.

Cys-Amadori (Path A) or the reaction of cysteine with the degradation products of Gly-Amadori (Path B) (de Roos, 1992; Gong et al., 2016). However, up to now, there is scarce information about how glycine competes with cysteine to react with reducing sugars in the initial stage of the Maillard reaction in terms of the formation of initial intermediate products and the pathways to yield meat-like flavors.

In this study, some reaction models composed of cysteine-xylose with different amount of glycine or without glycine were designed and performed, from which the effect of glycine on the cysteine-xylose reaction for development of meat-like flavors was discussed, based on the study of reaction progress and chemical profile of the initial intermediate products formed in the reaction mixtures, and comparison of amount of meaty flavor compounds produced in the reaction mixtures. The work is helpful to further understand how meat flavor is generated in a complex Maillard reaction system composed of cysteine as well as other amino acids, and can also provide some guidance for efficient preparation of processed-meat flavorings.

2. Materials and methods

2.1. Materials and reagents

L-Cysteine (99%), L-glycine (99%), D-xylose (99%), and n-alkanes ($C_6 \sim C_{26}$) were purchased from J&K Chemical Ltd. (Beijing, China). Besides, the authentic chemicals including 2-methylthiophene(99%), pyrazine(97%), 2-methylthiazole(98%), thiazole(99%), dihydro-2-methyl-3(2H)-furanone(98%), 2-furfurylthiol(97%), 2-methyl-3-furanthiol (97%), 3-mercapto-2-pentanone(98%), furfural(99%), 2-thiophenethiol (98%), 2-acetylthiazole(98%), 1-(3-thienyl)ethanone(99%), 3-(methylthio)thiophene(98%), dihydro-2-methyl-3(2H)-thiophenone(98%), 3,3'-dithiobis-(2-methyl)-furan(98%), 3-methyl-2-thiophenecarboxaldehyde(99%), and 2,3-dihydro-5-methylthiophene(98%), which were

used to identify the compounds in GC-MS, were also obtained from J&K Chemical Ltd. (Beijing, China). Ammonium formate, NaH_2PO_4 , and NaOH were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Ultrapure water was prepared by a Milli-Q water purification system (Millipore, Volketswil, Switzerland). The other chemicals used in this study were all of analytical grade.

2.2. Model reactions

The reactions were conducted in a 15 mL pressure glass vial (Beijing Synthware Glass Inc., China) on a Parallel Synthesis Poly-block 4 System (Hel Limited Co., England). The amounts of cysteine and xylose used in the reaction models were both 2 mmol in 5 mL of phosphate buffer (0.2 M, pH 5.5). The glycine was added in the reaction systems at 0, 1, 8, 12, 16, or 24 times of the cysteine concentration (mol/L). The vials were sealed and heated at 90 °C while stirring for 0.5, 1, 2, 3, 4, or 5 h. At the end time, the vials were removed and cooled immediately by a stream of tap water. All the reaction mixtures were subjected to HPLC-ELSD, HPLC-MS, UV/Vis, and pH analyses. Solid phase microextraction coupled to gas chromatography and mass spectrometry (SPME/GC-MS) was employed for the 3 h reaction mixtures to analyze volatile flavor compounds. Three replicates were performed for the reactions as well as the analyses.

2.3. Synthesis of Gly-Amadori, Cys-Amadori, and TTCA

Xylose-derived Gly-Amadori (*N*-(1-deoxy-D-xylulos-1-yl)-L-glycine) was prepared according to Hao et al. (2007). Xylose-derived Cys-Amadori (*N*-(1-deoxy-D-xylulos-1-yl)-L-cysteine) and TTCA (2-threitylthiazolidine-4-carboxylic acid), were prepared as described by de Roos et al. (2005), while the samples of reactions at 50 °C for 1 h were used

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