



Effects of ribose to cysteine ratios on the formation of volatile compounds from the Maillard reaction in supercritical carbon dioxide

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ABSTRACT

The volatile compounds formed from the Maillard reaction in a model system treated with/without supercritical carbon dioxide (SC-CO₂, 40 MPa, 140 °C) were investigated at three molar ratios (3:1, 1:1, and 1:3) of ribose to cysteine. The headspace volatiles generated in the reaction mixtures were examined by SPME in combination with GC–MS. The ratio of ribose to cysteine had complex effects on the volatiles formation and those effects got further complicated by the SC-CO₂ treatment. The formation of thiols and polysulfur heterocyclic compounds was generally favoured at an equal molar ratio of reactants; disulfides were favoured at low ratio of ribose to cysteine; while the effect of reactant ratios on the formation of thiophenes and fused bicyclic compounds did not follow a consistent pattern. SC-CO₂ treatment resulted in a reduction of most volatiles, however, promoted the formation of some well-known meaty aromatic compounds.

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1. Introduction

The Maillard reaction, which is manifested by the development of flavour and colour in many processed foods, is known to be highly pH, temperature, moisture, and reactant dependent. As reactant is concerned, different carbohydrates, amino acids, and their combinations result in different aroma profiles and reaction kinetics. Ribose and cysteine are important precursors of meat flavours, and the volatile compounds generated from ribose–cysteine model system have been investigated to a certain extent. However, few studies have systematically investigated the influence of reactant ratios on the formation of volatiles from Maillard reaction between cysteine and ribose. Hofmann and Schieberle (1995) evaluated the overall odour profiles of conventional thermally treated ribose–cysteine reaction mixtures with ratios of ribose to cysteine ranging from 1:1 to 10:1 at pH 5.0. They found that reactant ratios significantly affected the overall aromatic profiles, and the profile would be dominated by caramel-like and burnt aromas with increasing ratio of ribose to cysteine to 10:1. Their work was

much more meaningful for process flavours manufacturing, but the details of volatile composition affected by reactant ratios were not reported.

Supercritical carbon dioxide (SC-CO₂), a special state of carbon dioxide (CO₂), is an excellent alternative to conventional organic chemical solvents. SC-CO₂ processing has been available for many years as commercial scale plants using CO₂ have been brought on line in food ingredients industry. The role of CO₂ as a reactant or medium in green chemical reaction has also been investigated somewhat continuously in the past decades (Berkman, 2004). However, only a little attention was given on evaluating the SC-CO₂ effects on Maillard reactions. Yalpani (1993) found that a high amount of water soluble imine-linked, branched chitosan derivatives was converted in the SC-CO₂-treated mixtures of chitosan and glucose or malto-oligosaccharides. Casal, Ramírez, Ibañez, Corzo, and Olano (2006) stated that the lactosylation of β-lactoglobulin (β-Ig) or caseinmacropeptide (CMP) and formation of furosine were suppressed, when SC-CO₂ was applied to lactose-CMP/β-Ig static system at 30 MPa and 50 °C with different pH values. The special physicochemical properties of SC-CO₂ have attracted much attention, and the fact whether SC-CO₂ could be used to create new aromatic products is still veiled, which call for much investigation of the Maillard reaction in SC-CO₂.

The study reported here was designed to investigate the effects of reactant ratios of ribose to cysteine on the volatiles generated from aqueous ribose–cysteine model system with or without the application of SC-CO₂.

Abbreviations: SC-CO₂, supercritical carbon dioxide; SPME, solid-phase microextraction; GC–MS, gas chromatography mass spectrometry; TIC, total ion count; DVB/CAR/PDMS, divinylbenzene/Carboxen™/poly(dimethylsiloxane); RP-HPLC, reverse phase high performance liquid chromatography; HMF, 4-hydroxy-5-methyl-3-(2H)-furanone; MFT, 2-methyl-3-furanthiol; FFT, 2-furanmethanethiol; 3-TT, 3-thiophenethiol; 2-TT, 2-thiophenethiol; MTT, 2-methyl-3-thiophenethiol.

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2. Materials and methods

2.1. Chemicals

L-Cysteine (>98%) and sodium pyrophosphate tetrabasic (>99%) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sodium pyrophosphate dibasic, alkane standard solution C₈–C₂₀, tridecane, and 4-hydroxy-5-methyl-3(2H)-furanone were purchased from Sigma–Aldrich Chemical Co. (Shanghai, China). d-Ribose was purchased from Amresco Inc. (Ohio, USA). The authentic aromatic chemicals were purchased the highest purity grade available from Sigma–Aldrich (Shanghai, China).

2.2. Preparation of reaction mixtures

D-Ribose (0.1 and 0.033 M) or L-cysteine (0.1 and 0.033 M) in 0.2 M pyrophosphate buffer at pH 5.6 was prepared separately. Pyrophosphate buffer (0.2 M, pH 5.6) was prepared by mixing appropriate volumes of 0.2 M sodium pyrophosphate dibasic and 0.2 M sodium pyrophosphate tetrabasic. d-Ribose (7.506 and 2.502 g) or L-cysteine (6.058 and 2.019 g) was then dissolved in about 100 ml of the 0.2 M pyrophosphate buffer at pH 5.6. This usually caused a slight drop in pH, which was adjusted to 5.6 with 0.2 M sodium pyrophosphate tetrabasic. The solution was then made up to 500 ml with 0.2 M pyrophosphate buffer of the same pH.

To study the effect of reactant ratio on the volatile formation, the Maillard reaction was conducted at three reactant ratios: 3:1, 1:1 and 1:3. For experiments at the reactant ratio of 1:1, equal volumes (50 ml each) of 0.1 M d-ribose and 0.1 M L-cysteine were mixed together. For experiments at the ribose to cysteine ratios of 3:1 and 1:3, 50 ml each of 0.1 M and 0.033 M d-ribose was mixed with an equal volume of 0.033 M and 0.1 M L-cysteine, respectively. All the treatments, with or without SC-CO₂, were performed with a CWYF-2 apparatus (Fig. 1, Hua'an Petroleum Scientific Instrument Co., Nantong, China) as previously described (Xu, Gao, Liu, & Zhao, 2008). The reaction apparatus consisted of a two-plunger type pump, a 300 ml stainless steel reaction vessel, a temperature controller and displayer, two 300 ml absorption chambers, and four pressure gauges (with an accuracy of ±0.2 MPa). The reaction vessel was enveloped by an electrical heating jacket with a thermocouple attached to the bottom to monitor the temperature inside the vessel. Another thermocouple, connected to the temperature controller, was placed inside the heating jacket to control the vessel temperature. Reactant solutions were poured into the reaction chamber and mixed with

mechanical agitation. Carbon dioxide (>99.9%, Beijing Praxair Inc., China) was pumped into the chamber through an active carbon pre-column. The pressure inside the vessel was raised to 40 MPa while the temperature was raised to 140 °C through the heating jacket. The supercritical conditions were maintained for 1 h; after that, the reaction chamber was cooled to room temperature by pumping cold 30% ethanol (−3 °C) through the jacket. The chamber was then depressurized, allowing the carbon dioxide, which was loaded with aromatic compounds, to be released sequentially into two absorption chambers, each containing 50 ml of the same phosphate buffer used in the reaction mixture. The carbon dioxide was finally released through the outlet into another 50 ml of the same buffer to capture the remaining volatiles carried by the exhausting carbon dioxide. Reactions of ribose or cysteine alone, 50 ml of 0.1 M d-ribose or L-cysteine diluted with an equal volume of 0.2 M pyrophosphate buffer, were also carried out. The reaction mixtures and the three (50 ml each) absorption buffers were collected separately and stored below −18 °C until use.

2.3. Instrumental analysis

The volatile compounds in reaction mixtures as well as the three absorption buffers were analysed by headspace solid-phase microextraction in tandem with gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS) followed Xu et al. (2008). 4-Hydroxy-5-methyl-3(2 H)-furanone (HMF) was determined by an Agilent 1100 HPLC system equipped with a diode array detector and a ChemStation for data analysis. Separation of HMF was carried out on a Zorbax SB-C18 column (5 μm, 250 mm × 4.6 mm i.d., Agilent Inc.), which was guarded with a 10 mm SB-C18 pre-column, with a linear methanol/water gradient of 5–85% methanol over 30 min. The column was washed between runs with 100% methanol. Reaction mixtures were diluted with 10 times deionized water and filtered through 0.45 μm membrane, and the filtrations (20 μl) were injected to the column without further pre-treatment. Chromatograms were monitored at 280 nm, with raw spectral data collection between 190 and 600 nm. HMF was identified by direct comparison of its retention time with that of the authentic compound under identical conditions and by spiking the samples with the standard, and further confirmed following a procedure that first prepared with a semi-preparative Zorbax SB-C18 column (5 μm, 250 mm × 9.4 mm i.d., Agilent Inc.), then GC-MS analysis based on comparing authentic compound. The concentration of HMF was calculated from the calibration curve analysed under the same HPLC condition.

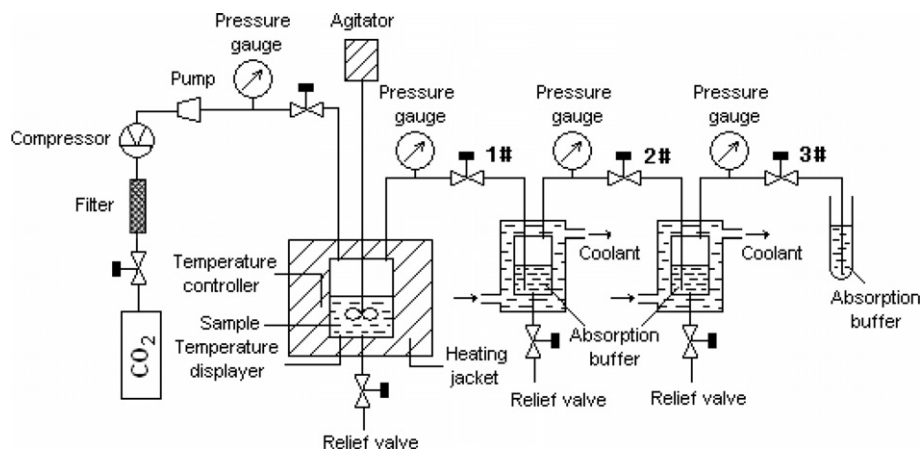


Fig. 1. Schematic diagram of the supercritical carbon dioxide reaction system.

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