High-intensity ultrasound production of Maillard reaction flavor compounds in a cysteine–xylose model system

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ABSTRACT

Application of high intensity ultrasound has shown potential in the production of Maillard reaction odor-active flavor compounds in model systems. The impact of initial pH, sonication duration, and ultrasound intensity on the production of Maillard reaction products (MRPs) by ultrasound processing in a cysteine–xylose model system were evaluated using Response Surface Methodology (RSM) with a modified mathematical model. Generation of selected MRPs, 2-methylthiophene and tetramethyl pyrazine, was optimal at an initial pH of 6.00, accompanied with 78.1 min of processing at an ultrasound intensity of 19.8 W cm^{-2}. However, identification of volatiles using gas chromatography–mass spectrometry (GC/MS) revealed that ultrasound-assisted Maillard reactions generated fewer sulfur-containing volatile flavor compounds as compared to conventional heat treatment of the model system. Likely reasons for this difference in flavor profile include the expulsion of H$_2$S due to ultrasonic degassing and inefficient transmission of ultrasonic energy.

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1. Introduction

Ultrasound is defined as any sound frequency above 20 kHz, i.e. above the threshold of human hearing [1]. The ability of ultrasonic irradiation to induce chemical reactions is widely regarded to be due to acoustic cavitation – the production of bubbles which eventually collapse to produce localized "hotspots". At high ultrasonic wave intensity, differences between the local pressure and the vapor pressure of the liquid develop. This produces new cavities, and enhances the bubble growth of gas nuclei originally present within the fluid. The bubble grows over several expansion and compression cycles until the oscillation of the bubble wall is equivalent to that of the applied ultrasound frequency [2–4]. Within this critical size range, the bubble collapses rapidly during a single compression cycle. The imploding bubble subjects the vapor contents and liquid–gas interface to high temperatures and pressures of approximately 5000 K and 1000 atm respectively. Other than enhancing mass transfer and compound degradation, the combined effects of the immense heat, pressure, and turbulence also include the induction of new reaction pathways or even the generation of products which are not formed under conventional conditions [5]. Besides creating "hotspots" of localized heat and pressure, this phenomenon can also generate highly reactive free radicals that promote chemical transformations [2].

Low- and high-intensity ultrasounds have very different applications in the industrial processing and production of foods. While low-intensity ultrasound (<1 W/cm^2, 5–10 MHz) is unable to physically or chemically alter the properties of a food material, high-intensity ultrasound (10–1000 W/cm^2, 20–100 kHz) can be used for freezing, sterilization, extraction, emulsification, and drying as alternatives for conventional treatments [2,6]. Some advantages of ultrasound irradiation over traditional heat processing include shorter processing time, lower energy consumption, reduced thermal damage, and less artifact formation [6,7].

More recently, high-intensity ultrasound has been proven to produce a variety of Maillard reaction products (MRPs), such as pyrazines, alkanes, and esters, from a glycine–glucose model food system [8]. It thus merits an in-depth study on the novel application of ultrasound in flavor generation.

The Maillard reaction is a non-enzymatic browning reaction between an amino acid and a reducing sugar, which can occur at room temperature but progresses faster with heating. Stemming from the fact that the Maillard reaction involves a complex series of reactions, depending on factors such as the type of the amino acid and sugar reacted, temperature, time, water activity, presence of oxygen, and other food components, the Maillard reaction is capable of producing a diverse range of different flavor profiles [9].

Sulfur-containing MRPs belong to a group of powerful aroma compounds which play a dominant role in the flavor of cooked meats despite being present in trace amounts. Examples of sulfurous volatiles which provide beefy notes include 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), and bis(2-methyl-3-furyl)disulfide (MFT–MFT) [10]. The 2 thiols, MFT and FFT, are exceptionally valuable odorants because of their extremely low odor threshold of approximately 5 ppt in air [11]. However, past research has shown that the yield of these meaty thiols in Maillard model systems is extremely low, with their quantities accounting for only 0.0005–0.0042% of the total volatile production [12].

Since the rate of Maillard reaction is inversely proportional to the size of the sugar molecule, pentoses generally react faster than hexoses [13]. Thus, the highly-reactive xylose was used for the model system in this study. Moreover, the selection of xylose as the starting sugar has been proven to produce the highest yields of MRPs with a meaty flavor with thermal processing [12].

In addition, the use of a sulfur-containing amino acid such as cysteine in this study allows the potential production of a wider range of odorants than a model food system deficient in sulfur. The motivation behind choosing cysteine over methionine was due to the abundance of well-established research linking its presence to the formation of commercially-valuable meaty odorants [14].

This study optimized the production of important MRPs under the following parameters: (1) initial pH, (2) length of exposure to ultrasound processing, and (3) ultrasound intensity (by altering amplitude) using Response Surface Methodology with a modified model. We also compared the differences in flavor generation when ultrasonic energy, instead of heat energy, was provided to a cysteine–xylose model food system.

2. Materials and methods

2.1. Materials

L-cysteine hydrochloride monohydrate of 99.0% purity and D-xylose of 99.7% purity were obtained from Wacker Chemicals (Singapore) and Danisco (Singapore) respectively. Analytical grade sodium dihydrogen phosphate (>99% purity) was from Sigma–Aldrich (Singapore), whereas food-grade anhydrous citric acid was acquired from Suntop (Singapore). An internal standard solution of 2,6-dichloroaniline (99.5% purity, Sigma–Aldrich, Singapore) dissolved in analytical grade absolute ethanol (Merck Millipore, Singapore) was used as well.

2.2. Methods

2.2.1. Preparation of the model food system

L-cysteine and D-xylose were dissolved in 60 mL of a phosphate-citric acid buffer (0.2 M and 0.1 M respectively) to make up a 0.100 mol dm^{-3} cysteine–xylose model food system. The pH was then adjusted with 0.2 M sodium dihydrogen phosphate or 0.1 M citric acid as required. Deionized water was added to bring the solution to 70 mL. The solution was then mixed thoroughly before subsequent ultrasound processing.

2.2.2. Processing of the model food system

2.2.2.1. Ultrasound processing. Using an ultrasound system (UIP 1000, Hielscher, Germany) with a Sonotrode BS2d18 (Hielscher, Germany) which operates at a frequency of 20 kHz, various intensities and treatment times of the ultrasound were performed on the cysteine–xylose solution at various initial pH. The ultrasound system was programmed to process the samples with a 5-s-on-and-5-s-off pulsation using the computer-supported control (UPCCTRL V3.2 WIN). For each trial, 70 mL of cysteine–xylose solution was placed in an amber glass container (diameter: 6.0 cm; height: 5.0 cm; wall thickness: 0.1 cm) for sonication. A thermocouple was used to record the temperature profile of the sample matrix throughout the Maillard reaction, so as to ensure the maintenance of a steady sample temperature of 60 ± 3 °C via an ice bath at 0 °C. The ultrasound probe of 1.8 cm in diameter was submerged 2.5 cm below the surface of the cysteine–xylose solution. Ultrasound processing was initiated after the container's opening was sealed with parafilm. Controls consisting of an identical cysteine–xylose solution were heated and then kept at 60 °C for the same treatment duration as the sonication. After ultrasonic or thermal treatment, the reaction vessel containing the solution was immersed in an ice bath until the mixture reached room temperature (i.e. 25 °C). To prevent the degradation of heat- and light-sensitive MRPs, the samples were kept in aluminum-wrapped screw-cap bottles at 4 °C before chemical analysis within 24 h. Response Surface Methodology (RSM) modeling tests were carried out in duplicates, whereas all other tests were conducted in triplicates.

2.2.2.2. Heat processing. For each trial, 70 mL of pH 5.0 cysteine–xylose solution was placed in a 200 mL screw-cap glass bottle, capped, then heated in an autoclave at 120 °C without stirring. The samples were taken out after heating for 60 min. After the heating period, the screw-cap bottles containing the solution were immersed in an ice bath until the mixture reached 25 °C. The samples were kept in aluminum-wrapped screw-cap bottles at 4 °C before chemical analysis within 24 h. Tests were conducted in triplicates.

2.2.3. Analysis of flavor compounds

2.2.3.1. Measurement of pH and UV–vis absorbance. An 827 pH lab meter (Metrohm AG, Switzerland) was used to determine the initial and final pH of the cysteine–xylose model food system.

The non-colored and non-fluorescent intermediates of the Maillard reaction were monitored by determining the UV–vis absorbance at 294 nm, A_{294} [15]. On the other hand, the degree of browning was studied by measuring the absorbance at 420 nm, A_{420}. This measure acts as an indicator for the overall progression of the Maillard reaction and symbolizes the advanced stage of the Maillard reaction [15]. Appropriate dilutions were made when necessary to obtain an optical density of less than 2, so as to not exceed the upper limit of the spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan).

2.2.3.2. SPME extraction of flavor compounds. The production of sulfurous MRPs from ultrasound processing of the cysteine–xylose model food system was analyzed with headspace solid-phase microextraction (SPME), coupled with gas chromatography–mass spectrometry (GC–MS).

For each sample, 4 mL of the processed solution was transferred to a 10-mL glass screw-thread vial (Agilent Technologies, CA, USA) containing 0.8 grams of NaCl salt. Vial headspace was extracted using divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CX/PDMS) SPME fiber with 50/30 μm DVB/CX on PDMS coating (Supelco Co., Bellefonte, PA, USA), with a manual holder (Supelco Co.). Sampling conditions for headspace extraction were optimized during preliminary trials. The sample vial was first incubated at 60 °C in a water bath for 10 min, with continuous agitation by a magnetic stir bar, for equilibration. The SPME fiber was then exposed to the headspace of the sample in the glass vial for a further 40 min, with constant agitation and incubation at 60 °C. Desorption of volatiles was performed by injecting the fiber in the injection port of the GC for 10 min.