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Investigation and kinetic evaluation of the reactions of hydroxymethylfurfural with amino and thiol groups of amino acids

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ABSTRACT

In this study, reactions of hydroxymethylfurfural (HMF) with selected amino acids (arginine, cysteine and lysine) were investigated in HMF-amino acid (high moisture) and Coffee-amino acid (low moisture) model systems at 5, 25 and 50 °C. The results revealed that HMF reacted efficiently and effectively with amino acids in both high and low moisture model systems. High-resolution mass spectrometry (HRMS) analyses of the reaction mixtures confirmed the formations of Michael adduct and Schiff base of HMF with amino acids. Calculated pseudo-first order reaction rate constants were in the following order; $k_{Cysteine} > k_{Arginine} > k_{Lysine}$ for high moisture model systems. Comparing to these rate constants, the $k_{Cysteine}$ decreased whereas, $k_{Arginine}$ and k_{Lysine} increased under the low moisture conditions of Coffee-amino acid model systems. The temperature dependence of the rate constants was found to obey the Arrhenius law in a temperature range of 5–50 °C under both low and high moisture conditions.

1. Introduction

Hydroxymethylfurfural (HMF) is a furanic aldehyde that is formed in food during thermal process via the Maillard reaction and from dehydration of sugars under acidic conditions (Ames, 1992; Kroh, 1994). HMF content of foods is mainly related to the thermal load applied during processing thus it is used as a marker for monitoring the thermal process (Gokmen & Acar, 1999; Ibarz, Pagan, & Garza, 2000; Morales & Jimenez-Perez, 2001; Rada-Mendoza, Sanz, Olano, & Villamiel, 2004). Besides, formation of HMF is dependent on pH and water activity (Ameur, Mathieu, Lalanne, Trystram, & Birlouez-Aragon, 2007; Morales, 2009). Coffee, fruit juices and breads are one of the most important sources of dietary HMF (Gokmen & Acar, 1999; Murkovic & Pichler, 2006).

HMF is a multifunctional molecule containing a furan ring, an unsaturated carbonyl group, and an allylic hydroxyl group that may undergo Schiff base formation with amines and Michael addition reactions in the presence of nucleophilic groups (Morales, 2009). In a study, Nikolov and Yaylayan (2011a) studied the reactivity of HMF with lysine, glycine and proline and the results of the study revealed the Schiff base formation in the presence of amino acids. In addition, the results of the study of Gokmen, Kocadagli, Goncuoglu, & Mogol, 2012 revealed that, carbonyl group of HMF rapidly reacted with amino acid asparagine leading to acrylamide formation through Schiff base formation. Likewise, in a recent study, cysteine alone or in combination with glycine significantly reduced the HMF content by forming Michael adducts in asparagine/glucose model as well as in biscuits (Zou et al., 2015).

Although it is known that HMF is a reactive compound, there are limited data about the reactions of HMF with amino acids and its kinetic behaviour. To date, the effect of the amino acids on HMF elimination have been studied in model systems to mitigate HMF formation during heating at elevated temperatures. To the best of our knowledge, there is no data on the reactions of HMF with amino or thiol groups take place at storage or physiological conditions. The elimination of HMF through its reactions with amino acids under these conditions is of importance from a viewpoint of food quality and safety. This study aimed to investigate the reactions of HMF with amino and thiol groups of amino acids at relatively low temperatures (≤ 50 °C) under both low and high moisture conditions. High-resolution mass spectrometry (HRMS) was used to identify and confirm the reaction products of HMF with amino acids (Michael adducts and Schiff bases) using HMF-amino acid and coffee-amino acid model systems. Reaction kinetics and its temperature dependence were also investigated.

2. Experimental

2.1. Chemicals and consumables

HMF, L-cysteine (> 99%), L-Lysine, L-arginine were purchased from

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Sigma-Aldrich (Steinheim, Germany). Formic acid (98%), methanol and acetonitrile were from JT Baker (Deventer, Holland). Potassium hexacyanoferrate, zinc sulfate, disodium hydrogen phosphate anhydrous, and sodium dihydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany). The Carrez I and Carrez II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 ml of water, respectively. Syringe filters (nylon, 0.45 μm) and Atlantis dC18 (250 × 4.6 mm, 5 μm) column were supplied by Waters (Millford, MA).

2.2. Preparation of model systems

Model systems were prepared to monitor the changes in the concentrations of HMF during reaction with different amino acids under high and low moisture conditions. For the preparation of high moisture model systems, 8 μmoles of HMF and 30 μmoles of amino acids were dissolved in 1.5 mL of 0.05% benzoic acid solution. Since HMF content of foods is minor compared to amino acids and not to limit the possible reactions of HMF with amino acids, excess amount of amino acids was added to reaction medium. Benzoic acid was used in order to prevent microbial growth during the reaction period. The pH of these model systems was measured as 3.5. "HMF" model system without the addition of amino acid was prepared to monitor the self-degradation of HMF during storage. Arginine, cysteine and lysine were added to HMF solution to prepare "HMF-Arg", "HMF-Cys" and "HMF-Lys" model systems, respectively.

Roasted coffee was selected to simulate the low moisture conditions to investigate the reactions of HMF with amino acids. Green coffee beans were roasted in an oven at 220 °C for 10 min. This condition was selected since it cause to the formation of HMF at highest amounts in roasted coffee (Kocadağlı, Göncüoğlu, Hamzalıoğlu, & Gökmen, 2012). One gram of ground-roasted coffee was put into a glass flask. A total of 8 μmoles arginine, cysteine or lysine was added to prepare "Coffee-Arg", "Coffee-Cys" or "Coffee-Lys" model systems, respectively. Ground roasted coffee alone, as "Coffee" model system was also used to monitor the reactions of HMF with amino acids.

The flasks containing the mixtures of model systems were placed in water bath set at 5, 25 and 50 °C to monitor the reactions. These conditions were selected to reveal the reactions of HMF with amino acids at cold and high storage conditions besides the body conditions. Changes in the concentrations of HMF were determined at the end of 1, 2, 3, 5 and 7 days by means of high performance liquid chromatography (HPLC). Reaction products of HMF with amino acids were also determined by means of HRMS.

2.3. Analysis of HMF in the model systems by HPLC

Sample (1 mL) withdrawn from high moisture model systems was centrifuged at 11,180 × *g* for 5 min. Supernatant was filtered through 0.45 μm syringe filters into an autosampler vial. Sample (1*g*) withdrawn from low moisture model systems was triple extracted with water (5–2.5–2.5 mL) by vortexing for 3 min. The combined extracts were clarified by adding 100 μl of Carrez I and Carrez II solutions. After vortexing for 30 s, the mixture was centrifuged at 10,000 × *g* for 5 min. Supernatant was filtered through 0.45 μm syringe filters into an autosampler vial.

The clear extract was injected onto a Shimadzu HPLC system (Kyoto, Japan) consisting of a quaternary pump, an autosampler, a diode array detector and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column using the isocratic mixture of 10 mM aqueous formic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 mL/min as the mobile phase at 25 °C. Data acquisition was performed by recording chromatograms at 285 nm. Concentration of HMF in dry basis was calculated by means of a calibration curve built in the range between 0.5 and 50 μg/mL (0.5, 1, 2, 5, 10 and 50 μg/mL).

2.4. Analysis of the reaction products of HMF with amino acids in the model systems by HRMS

Sample (1 mL) withdrawn from high moisture model systems was centrifuged at 11,180 × *g* for 5 min. Supernatant was filtered through 0.45 μm syringe filters into an autosampler vial. Sample (1 g) withdrawn from low moisture model systems was triple extracted with water (5–2.5–2.5 mL) by vortexing for 2 min. The combined extract was centrifuged at 11,180 × *g* for 5 min. Supernatant was filtered through 0.45 μm syringe filters into an autosampler vial.

To identify and confirm the reaction products of HMF with amino acids, the clear extract was injected onto a A Thermo Scientific Dionex Ultimate Rapid Separation RSLC system coupled to a Thermo Scientific Q Exactive Orbitrap HRMS system. The HRMS system was operated in positive electrospray ionization mode. The chromatographic separations were performed on Atlantis Hypersil-gold column (100 mm 2.1 mm i.d., 1.9 mm) by using a gradient mixture of 0.05% aqueous formic acid and methanol as the mobile phase at a flow rate of 0.5 mL/min (30 °C). The mobile phase gradient was programmed as follows: 70% of methanol for 6 min, linear increase to 95% of methanol within 3 min, 95% of methanol for 3 min, and linear decrease to 70% of methanol within 3 min. The scan analyses were performed in a *m/z* range between 50 and 600 at ultra-high resolving power (R = 100,000). The data acquisition rate, the automatic gain control target and maximum injection time were set to 1 Hz, 1×10^6 and 100 ms, respectively. The source parameters were as follows: sheath gas flow rate 30 (arbitrary units), auxiliary gas flow rate 10 (arbitrary units), discharge voltage 4.5 kV, discharge current 5 μA, capillary temperature 330 °C, capillary voltage 47.5 V, tube lens voltage 115 V and vaporizer temperature 330 °C. The corresponding ions were extracted from the total ion chromatograms to confirm the reaction products in the extracts of model systems.

2.5. Statistical analysis

The data were subjected to analysis of variance (one-way ANOVA). The SPSS 17.0 statistical package was used for the evaluation of statistical significance of the differences between mean values by Duncan test. $P < 0.05$ was considered to be statistically significant for the results.

3. Results & discussion

3.1. Changes in the concentrations of HMF in the model systems

Changes in the concentrations of HMF were monitored in both high and low moisture model systems kept at 5, 25 and 50 °C during the reaction period up to 7 days. Initial HMF content of roasted coffee was found to be 1.706 ± 0.061 μmoles. At the end of 7th day, 24.5% of HMF was depleted in coffee stored at 5 °C, whereas percentage depletion increased in the presence of amino acids. It was found that, 52.8%, 42.7% and 36.1% of HMF was depleted with the addition of amino acids in "Coffee-Arg", "Coffee-Cys" and "Coffee-Lys" model systems, respectively (Fig. 1a). As temperature is increased to 25 °C, elimination ratio gradually increased (Fig. 1b). When the temperature is 50 °C, this ratio reached to 39.7%, 57.6% 58.0% and 62.1% in "Coffee", "Coffee-Arg", "Coffee-Cys" and "Coffee-Lys" model systems, respectively (Fig. 1c).

In high moisture model systems, at the end of reaction period (7 days) at 5 °C, self-degradation of HMF was found to be 0.9% and as in HMF model systems, the elimination ratio increased in the presence of amino acids (Fig. 2a). Addition of cysteine caused to 41% depletion even at low temperature, 5 °C, whereas almost all HMF was eliminated at the end of reaction period at 50 °C (97%). Percentage depletion in HMF was observed respectively as 9.7%, 52.8% and 6.5% for "HMF-Arg", "HMF-Cys" and "HMF-Lys" model systems reacted at 25 °C for