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# Improved controlled flavor formation during heat-treatment with a stable Maillard reaction intermediate derived from xylose-phenylalanine

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ABSTRACT

The Maillard reaction intermediate (MRI) and Maillard reaction products (MRPs) derived from xylose (Xyl) and phenylalanine (Phe) were prepared, then stored at 25 °C for 60 days. After storage, the contents of flavor compounds and the clarity of MRPs solution decreased, and the apparent Z-average hydrodynamic diameter ($D_h$) of particles in the solution increased from 149 to 439 nm. However, the MRI solution remained transparent during storage. The concentration of MRI only decreased by 6.49%, and $A_{294}$ of the solution increased slightly yet $A_{420}$ remained stable. Numerous flavor compounds in MRPs decreased during heat treatment, meanwhile the cross-linking and aggregation of MRPs were intensified, and the particles' $D_h$ increased to micron level. The heated MRI solution showed a similar appealing profile and flavor fingerprints as the MRPs solution before heat treatment. Controlled formation of flavors from MRIs is proposed to be used as potential alternative to the existing Maillard flavorings.

## 1. Introduction

The most important parameter that maximizes food quality and global competitiveness is flavor (Weerawatanakorn, Wu, Pan, & Ho, 2015). Flavorings are constantly used to impart taste and smell, and to intensify the existing flavors of food products (Kaitano, 2014). They are considered as effective food additives for savory flavor enhancement (Methven, 2012). Maillard reaction is of utmost importance for food flavor formation during heat treatment (Hou et al., 2017). Maillard reaction is initiated by the condensation of reducing sugars and amides, which leads to the formation of a labile *N*-substituted amino sugar. Through dehydration of *N*-substituted amino sugar, Schiff base is generated (Troise, Berton-Carabin, & Fogliano, 2016). Subsequently, rearrangement of the Schiff base occurs via 1,2-eneaminol resulting in Amadori rearrangement products (ARPs) (Troise et al., 2016). ARPs are relatively stable Maillard reaction intermediates (MRIs) produced during the initial stage of Maillard reaction (Harohally, Srinivas, & Umesh, 2014). They are significant non-volatile flavor precursors, and tend to generate the end-products during the thermal reaction (Harohally et al., 2014).

Maillard reaction products (MRPs) have great appeal to consumers for decades and have intrigued researchers because of their strong fragrance (Parker, 2015). Many of Maillard key flavor compounds such as furan and pyrrole etc., are prepared for food additive through chemical synthesis (Deblander, Van Aeken, Adams, De Kimpe, & Abbaspour Tehrani, 2015). However, MRPs are highly susceptible to flavor loss during storage and processing due to their unstable physicochemical properties (Kaitano, 2014), MRPs such as pyranone and unsaturated diones are documented to be the most prone to degradation (Andrewes, 2012). Nowadays, the flavor loss and deterioration are realized as important problems during the processing and storage of foods or flavorings (Jansen, 2015), especially at temperatures higher than room temperature (He, Qian, & Qian, 2018). Thus, an increasing attention is being given to the stability of flavor and flavorings (Weerawatanakorn et al., 2015), since the loss of desirable aroma components such as acetaldehyde, butanal, furfural etc., diminishes both flavor quality and consumer acceptability (Hansen & Arora, 1990).

Tremendous work in the stabilization of the flavor compounds in MRPs during storage and processing was done during the past decades (Asikin et al., 2014; Lotfy, Fadel, El-Ghorab, & Shaheen, 2015), and suggested encapsulation as an efficient method for the stabilization of flavors (Ghajari, Iman, Mohammad, & Mahdi, 2017). There is also

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considerable interest in the potential of stable flavor precursors, the MRIs, to extend the shelf-life of flavorings and to form fresh flavor when required, based on their reactivity and aroma generation. A growing body of evidence suggested the decomposition of MRIs during heat treatment as Maillard reaction. Heterocyclic compounds and reductones have been reliably produced from fission reaction and dehydration of MRIs (Coleman & Chung, 2002), and abundant flavor compounds and melanoidins that give food an appealing dark color and desirable aroma are formed from such reactions (Coleman & Chung, 2002; Cui et al., 2017). Thus, the MRIs have the potential to be excellent substitutes of MRPs, and the flavor formation can be achieved with a high level of control. As reactants of Maillard reaction, amino acids and sugars are stable precursors of the flavors, however, much higher reactivity of MRIs was observed to generate browning pigments and flavor compounds compared to the amino acids and sugars (Cui et al., 2017). Therefore, the idea that MRIs are a great candidate to allow the generation of fresh flavor for use in unprocessed or minimally processed food products was proposed. The fresh flavors would not only provide the characteristic aroma of the corresponding MRPs but also become the carriers of hedonic information that foster cooking motivation of food consumers (Prescott, 2016). Thus, the controlled formation of flavors derived from MRIs can have significant and revolutionary advances in the area of Maillard flavorings.

Sufficient flower-like flavor compounds could be formed during the Maillard reaction of xylose (Xyl) and phenylalanine (Phe), and the MRPs could be important in dessert application (Cui et al., 2017; Samakradhamrongthai, Thakeow, Kopermsub, & Utama-Ang, 2017). In this research, the MRI and MRPs derived from Xyl-Phe were prepared in aqueous medium, and stored at 25 °C for 60 days. The changes in profile and concentration of MRI were monitored during storage. The apparent Z-average hydrodynamic diameter ($D_h$) of particles and flavor profile of the MRPs before and after storage were analyzed. The storage stability advantage of MRI was confirmed. Meanwhile, the MRI and MRPs solutions were heated to generate flavor compounds. The flavor profiles and visible characterization of their final products were analyzed and compared. The Xyl-Phe derived MRI was confirmed to be used as potential alternative to the existing Maillard flavorings, and a controlled way to create flavor products using MRI as a means to improve the efficiency of this process was proposed.

## 2. Materials and methods

### 2.1. Materials and reagents

D-xylose, anhydrous ethanol, L-phenylalanine, 2,3,5-triphenyltetrazoliumchloride and *n*-alkanes ($C_6$-$C_{26}$) were provided by Sigma-Aldrich Chemical Co. (Shanghai, China). Formic acid and acetonitrile were provided by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). 1,2-Dichlorobenzene (98%) was provided by Macklin Biochemical Co. (Shanghai, China). Standard ARP (*N*-(1-deoxy-α-D-xylulos-1-yl)-phenylalanine) (98%) was prepared in our lab using the protocol and procedures defined in the Sections 2.2 and 2.3.

### 2.2. Preparation of the MRI

The MRI was prepared according to the reported procedures (Cui et al., 2017) with some modifications. Briefly, Xyl and Phe mixture (5 g) in the molar ratio of 2:1, dissolved in deionized water (80 mL), was adjusted to a pH of 7.4 using NaOH aqueous solution (3 mol/L). This solution was heated in water bath for refluxing at 80 °C for 50 min, and then the atmospheric pressure inside the bottle was decreased to 25 mbar in 5 min using rotary evaporator under vacuum (R-215, Büchi, Switzerland). The dehydration was conducted for facilitating the Xyl-Phe conversion to the MRI. After dehydration for 20 min, the mixture was immediately cooled in ice water. The obtained solid product was dispersed in anhydrous ethanol (40 mL), and rotary evaporated under vacuum at 30 °C for 30 min (the vacuum pressure was above 0.07 MPa). The objective of sample dispersion in anhydrous ethanol was to form azeotropic system of water-ethanol, so that the residual water could be removed at low boiling point using rotary evaporation, to stabilize the prepared *N*-(1-deoxy-α-D-xylulos-1-yl)-phenylalanine. The yield of the MRI was 47% measured by the method in Section 2.7.

### 2.3. Purification of the MRI

The MRI purification was performed referring to previously reported method (Cui et al., 2017; Davidek, Kraehenbuehl, Devaud, Robert, & Blank, 2005). The reaction solution was dried at 40 °C using rotary evaporator, and the obtained dry matter was mixed with 500 mL anhydrous ethanol. The undissolved components were filtered out and the solution was dried using a rotary evaporator. Next, the remaining solid of MRI was dissolved in deionized water. A column chromatography was used for the MRI purification, and the exchange resin of Dowex 50WX4 ion ($H^+$) was selected as the filler. Water was used for the elution until the eluent tested negative for 2,3,5-triphenyltetrazoliumchloride (TTC) test. Then the MRI was eluted with 0.2 mol/L ammonium hydroxide. A semi-preparative RP-HPLC (Waters, Milford, MA, USA) was used for further purification of the MRI. The sample was eluted at 1.0 mL/min through a linear gradient from 98% to 0 of phase A (formic acid, aq) with phase B (acetonitrile) for 20 min and the column of RP-HPLC $C_{18}$ (10 μm, 22 × 200 mm) was selected. The purified MRI was characterized by UPLC-MS-MS (Waters, Milford, MA, USA), and NMR (Bruker Bio Spin, Germany; 298 K) (Cui et al., 2017). The mass spectra (Fig. S1), NMR spectra (Fig. S2), and the chemical shifts ($\delta$ values) of $^1$H NMR and $^{13}$C NMR spectra are presented in supplementary data. According to the analysis results, the MRI derived from xylose-phenylalanine was identified as *N*-(1-deoxy-D-xylulos-1-yl)-phenylalanine (Cui et al., 2017).

### 2.4. Preparation of the MRPs

The MRPs derived from Phe and Xyl was prepared according to the method reported by Cui et al. (2017). Xyl and Phe were dissolved in water (the molar ratio of Phe to Xyl was 1:2) at the concentration of Phe 4.5 mmol/L. The solution was adjusted to the pH 7.4 and heated at 120 °C for 120 min for the formation of sufficient flavor compounds. In order to reach 120 °C of the reaction system, the reaction was in a temperature and pressure resistant bottle to keep a closed system. Then, the reaction was stopped by cooling in ice water.

### 2.5. Storage of the MRI and MRPs

Equal amounts of the MRI and MRPs were placed in the same type of sample container with capping. The sample containers were wrapped in aluminium foil to exclude light and stored together in an incubator (Jinghong, Shanghai, China) at 25 °C for 60 days. The MRI samples were removed from the incubator periodically and analyzed for the concentration of MRI (Section 2.7) and browning intensity (Section 2.8). The MRI solution kept pellucid during the storage while the MRPs solution became turbid. Thus, the MRPs samples were analyzed for particle size (Section 2.9) as well as flavor profile (Sections 2.10 and 2.11).

### 2.6. Thermal treatment of the MRI and MRPs

The MRI aqueous solution (4.5 mmol/L) was prepared. The solution was adjusted to a pH of 7.4 using 3 mol/L NaOH solution. Then, the solutions of MRPs and MRI were respectively heated in temperature and pressure resistant bottles at 100 °C, to keep closed systems during the thermal treatment. After the heat treatment for 120 min, the bottles with the reaction solutions inside were cooled immediately by ice water.