Food Chemistry 169 (2015) 224–229

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Separation and determination of 4-methylimidazole, 2-methylimidazole and 5-hydroxymethylfurfural in beverages by amino trap column coupled with pulsed amperometric detection

Xian-Bing Xu [a], Ding-Bo Liu [a], Shu-Juan Yu [a,b,c,*], Pei Yu [a], Zhen-Gang Zhao [a]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China
[b] Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou 510640, China
[c] State Key Laboratory of Pulp and Paper Engineering, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 22 June 2014
Accepted 30 July 2014
Available online 8 August 2014

Keywords:
4-Methylimidazole
2-Methylimidazole
5-Hydroxymethylfurfural
Solid-phase extraction
Beverage
AMTC-PAD
Caramelization products

ABSTRACT

A method for simultaneous determination of 4-methylimidazole (4-MeI), 2-methylimidazole (2-MeI) and 5-hydroxymethylfurfural (HMF) in beverages was developed using solid-phase extraction (SPE) and amino trap column coupled with pulsed amperometric detection (AMTC-PAD). A single amino trap column (P/N: 046122) was first applied to separate the targeted analytes in samples after SPE pretreatment. This method demonstrated low limit of quantification (0.030 mg/L for methylimidazoles and 0.300 mg/L for HMF) and excellent linearity with correlation of determination (R^2 = 0.999 for 2-MeI, 0.997 for 4-MeI and 0.998 for HMF). Nearly no 2-MeI was found in all soft drinks. However, 4-MeI could be detected in cola drinks and soft drinks containing caramel colour (ranging from 0.13 to 0.34 mg/L), whereas HMF were only found in cola drinks (ranging from 1.07 to 4.47 mg/L). Thus, AMTC-PAD technique would be a valid and inexpensive alternative to analysis of 4-MeI, 2-MeI and HMF.

1. Introduction

HMF, 4-MeI and 2-MeI were undesired byproducts produced during the manufacture of caramel colour (Capuano & Fogliano, 2011; Guan, Shi, Yu, & Xu, 2011; Guan, Wang, Yu, Yu, & Zhao, 2012; Hengel & Shibamoto, 2013; Moon & Shibamoto, 2010). HMF as a potential carcinogen was originated from the degradation of carbohydrate by the caramel reaction or Maillard reaction (Fig. 1) (Capuano & Fogliano, 2011; Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010; Guan et al., 2011; Islam, Khalil, Islam, & Gan, 2014; Martins & Van Boekel, 2005). Methylimidazoles, formed from the reaction between dicarbonyl compounds (methylglyoxal or glyoxal) and ammonia in Maillard reaction (Fig. 1) (Jang, Jiang, Hengel, & Shibamoto, 2013; Lee, Jang, & Shibamoto, 2013), were confirmed as harmful compounds at a high dose exposure. Hengel and Shibamoto (2013) reported that the addition of caramel colour into beverages or soy sauces led to increase HMF, 4-MeI and 2-MeI concentration levels. Consequently, a great deal of attention was paid to the toxic, mutagenic, and carcinogenic effects of these compounds in foods (Sengar & Sharma, 2012). Thus, a quantitative and routine analysis of HMF, 4-MeI and 2-MeI would be required for risk assessments and reliable exposure.

Traditionally, the separation and analysis of these polar compounds was accomplished by gas–liquid chromatography (GC) or reversed-phase liquid chromatography (RP-HPLC) method. For example, the acetyl derivatization of 4-MeI and 2-MeI were detected by a GC method (Casal, Fernandes, Oliveira, & Ferreira, 2002; Fuchs & Sundell, 1975). The determination of 4-MeI and 2-MeI in real foods were also performed by RP-HPLC method based on ion-pairing agent (Jang et al., 2013; Thomsen & Willumsen, 1981; Yamaguchi & Masuda, 2011). In addition, the determination of HMF was mainly carried out by RP-HPLC method (Delgado-Andrade et al., 2010). Wang and Schnute (2012) developed an improved method by an ultrahigh-performance liquid chromatography (UHPLC) tandem mass spectrometric (MS/MS) for the simultaneous quantification of 2-acetyl-4-tetrahydroxybutylimidazole (THI), 2-MeI, 4-MeI, and HMF in beverage samples, but the expensive instruments were not suitable for a small laboratory. Although these above-mentioned methods were applicable, stable and acute, a suitable modification of mobile phase or pre-column derivatization was required for the separation of polar compounds on GC and RP-HPLC column. Thus, a rapid and simple method for the routine

* Corresponding author at: College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China. Tel./fax: +86 20 87113668.
E-mail address: shujuanyu8@gmail.com (S.-J. Yu).

http://dx.doi.org/10.1016/j.foodchem.2014.07.149
0308-8146/

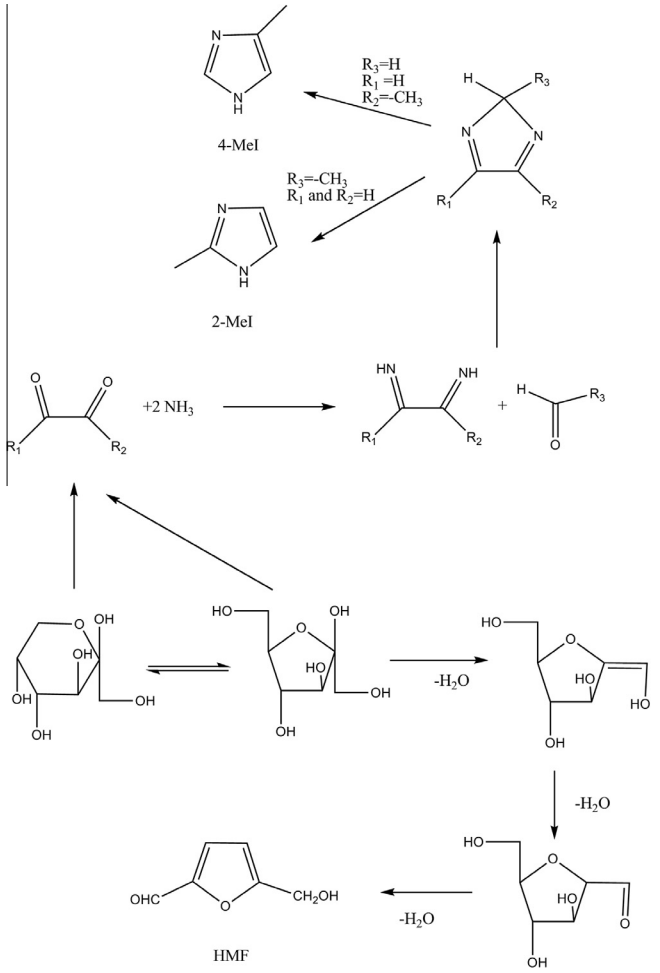

Fig. 1. Proposed mechanisms and the structures of 2-methylimidazole, 4-methylimidazole and 5-hydroxymethylfurfural formation.

analysis of 4-MeI and HMF was required. Methodologically, ion exchange chromatography, as a novel analytical technique first proposed by Small, Stevens, and Bauman (1975), was an ideal tool for the separation of ionic compounds or polar compounds without modification of mobile phase or pre-column derivatization. In this experiment, the target analytes as polar compounds (pKa values of the imidazole ring and HMF were 7.70 and 12.82, respectively.) were transformed into anionic forms in alkaline solution (Liu & Tan, 2013; Petruci, Pereira, & Cardoso, 2013). Thus, the separation of 4-MeI, 2-MeI and HMF by anionic exchange chromatography method should be an optional strategy. However, to the best of our knowledge, the simultaneous separation of 4-MeI, 2-MeI and HMF as ionic forms was nearly no reported in literatures.

This study aimed to develop an applicable method for the simultaneous quantitation of HMF, 4-MeI and 2-MeI after solid-phase extraction (SPE) in real food system. The method was accomplished by a single amino trap column coupled with pulsed integrated amperometric electrochemical detector (AMTC-PAD). The results were discussed on selectivity, linearity, accuracy and precision of the method.

2. Materials and methods

2.1. Chemical and reagents

4-MeI (99%), 2-MeI (99%), 1, 2-dimethylimidazole (1,2-MeI, 99%), sodium hydroxide (50%) and HMF (98%) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Methanol, ammonia and hydrochloric acid (HCl, 35–37%) were of analytic grade and purchased from Merck (Damstadt, Germany). The mixed standard stock solution of HMF (100 mg/L), 4-MeI (10 mg/L) and 2-MeI (10 mg/L) was prepared by dissolving the analytes into deionized water.

2.2. Samples preparation

The samples of cola and beverages were bought from local shops at Guang Zhou city. Soft drinks (a) with caramel colour and soft drink (b) and (c) without caramel colour had low levels of sugar. All the cola drinks contained caramel colour and only cola drink (e) did not contain sugar. Samples were degassed by a magnetic stirrer at room temperature for 30 min. A portion of drink (3 mL) was sampled before SPE. Class III caramel food colour (0.5 g/mL) was prepared and used as control to determine HMF, 4-MeI and 2-MeI.

2.3. Pretreatment of samples using SPE

Ansys SPEC SCX Disc 15 mg/3 mL cartridge (Varian, Walnut Creek, CA) was conditioned with 1 mL of methanol and 1 mL of water. The 3 mL samples (spiked 0.3 mg/L of 1,2-MeI as internal standard) acidified with 20 μL of HCl (0.1 mol/L) were loaded and passed through the cartridge. The retained imidazoles and HMF were eluted out with 6 mL of a methanol/ammonia (5%, v/v). The collected extract was evaporated to dryness at 39 °C and then the residues were dissolved with 3 mL deionized water for AMTC-PAD analysis.

2.4. AMTC-PAD conditions

AMTC-PAD was used for analysis of HMF, 4-MeI and 2-MeI. The samples were filtered through a Millex-HN nylon clarification kit of 0.45 μm pore size (Millipore, Bedford, MA), and analysed on a DX 5000 Dionex system (Dionex Corp., Sunnyvale, CA), a gradient pump (model EG40) with on-line degassing, and a pulsed integrated amperometric electrochemical detector (PAD, ED40, Dionex Corp., Sunnyvale, CA). Separation was accomplished on an amino trap column (4 × 50 mm, P/N: 046122) using the isocratic elution (100 mmol/L NaOH) for 60 min. All tests used a constant flow rate of 0.25 mL/min. The injection volume was 25 μL. The pH reference electrode (P/N: 061879) and gold working electrode (P/N: 061875) were used in AMTC-PAD analysis. The waveforms were applied under the following settings: $E_1 = 0.13$ V ($t_1 = 0.04$ s), $E_2 = 0.33$ V ($t_2 = 0.16$ s), $E_3 = 0.55$ V ($t_3 = 0.24$ s), $E_4 = 0.33$ V ($t_4 = 0.09$ s), $E_5 = -1.67$ V ($t_5 = 0.01$ s), $E_6 = 0.93$ V ($t_6 = 0.01$ s), $E_7 = 0.13$ V ($t_7 = 0.01$ s). Integration occurred from 0.20 to 0.44 s during E_3 application.

The calibration curves were listed in Table 1. The regression equation for HMF was $y_1 = 2.493x_1 + 3.891$; $R^2 = 0.998$, where x_1 = HMF concentration, mg/L; y_1 = peak area of HMF. The regression equation for 4-MeI was $y_2 = 16.480x_2 + 0.479$; $R^2 = 0.997$, where x_2 = 4-MeI concentration, mg/L; y_2 = peak area of 4-MeI. The regression equation for 2-MeI was $y_3 = 9.354x_3 + 0.470$; $R^2 = 0.999$, where x_3 = 2-MeI concentration, mg/L; y_3 = peak area of 2-MeI.

According to Sistla, Tata, Kashyap, Chandrasekar, and Diwan (2005), limit of detection (LOD) and limit of quantification (LOQ) was determined as three times and ten times observed signal-to-noise (S/N), respectively.

2.5. Recovery tests

The recoveries were calculated by comparing the peak areas of the spiked and non-spiked samples with the peak areas of standard