Short communication: Amino trap column improves the separation of methylimidazoles, 5-hydroxymethyl-2-furaldehyde, and sugars in Maillard reaction

Xian-Bing Xu,* Ding-Bo Liu,* Shu-Juan Yu,*†‡¹ Zhen-Gang Zhao,* and Pei Yu*

*College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China

†Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou 510640, China

‡State Key Laboratory of Pulp and Paper Engineering, Guangzhou 510640, China

ABSTRACT

A simultaneous analysis of methylimidazoles, reducing sugars, and 5-hydroxymethyl-2-furaldehyde in the Maillard reaction was improved by use of an amino trap column. Analysis was carried out by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) coupled with an amino trap column. The amino trap column was a useful tool to improve the separation of methylimidazoles, reducing sugars, and 5-hydroxymethyl-2-furaldehyde. This technique is useful for simultaneous analysis of methylimidazoles, reducing sugars, and 5-hydroxymethyl-2-furaldehyde in risk assessment for dairy products.

Key words: 4-methylimidazole, high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), Maillard reaction, reducing sugar, 5-hydroxymethyl-2-furaldehyde

Short Communication

The Maillard reaction, also known as the browning reaction, occurs in thermal food processing (Chappel and Howell, 1992; Leonard, 1997; Manzocco et al., 2000). Generally, the Maillard reaction involves the reaction between reducing sugars and amino compounds, which can be described into 3 stages: the early, advanced, and final Maillard reaction steps (Martins et al., 2000; Martins and Van Boekel, 2005; Sandwick et al., 2005). Consequently, a numerous compounds are generated in these stages, including 5-hydroxymethyl-2-furaldehyde (HMF; Göğüş et al., 1998; Capuano and Fogliano, 2011) and methylimidazoles (Lee et al., 2012). 5-Hydroxymethyl-2-furaldehyde is known as the degradation product of Amadori rearrangement products via enolization and dehydration reactions (Cammerer

et al., 1999; Guan et al., 2012a). In addition, methylimidazoles such as 4-methylimidazole (**4-MeI**) and 2-methylimidazole (**2-MeI**) are formed via the reaction between ammonia and α -dicarbonyls (methylglyoxal and glyoxal), which are formed via the degradation reaction of carbohydrates (Hengel and Shibamoto, 2013; Jang et al., 2013).

In dairy thermal processes, because of the presence of reducing sugars (lactose and glucose) and amino compounds (including amino acids and proteins), HMF and methylimidazoles are eventually produced via Maillard reaction (Moon and Shibamoto, 2011; Guan et al., 2012b). In addition, methylimidazoles can be introduced into the milk of cows fed ammoniated forage as a result of the interaction of ammonia with reducing sugars (Waagepetersen and Thomsen, 1977). Recently, some studies have reported that potential toxic, mutagenic, and carcinogenic effects were observed after dietary exposure to HMF (Guan et al., 2012c). Meanwhile, 2- and 4-MeI were confirmed as carcinogenic compounds on the basis of animal studies reported by the National Toxicology Program (NTP, Research Triangle Park, NC) and other researchers (Hengel and Shibamoto, 2013; Petrucci et al., 2013). Thus, the presence of these harmful compounds (HMF and methylimidazoles) in dairy products indicates that a reliable method for HMF and methylimidazoles analysis should be developed for risk assessment of dairy products.

Traditionally, the separation and analysis of methylimidazoles and HMF was accomplished by reversed-phase (RP)-HPLC or gas-liquid chromatography methods (Wilks et al., 1973; Fuschs and Sundell, 1975; Guan et al., 2011; Yamaguchi and Masuda, 2011). However, these methods are not suitable for separation of reducing sugars in samples. Thus, the simultaneous analysis of 4-MeI, 2-MeI, reducing sugar, and HMF by RP-HPLC was impossible. Recently, ion-exchange chromatography as a novel separation technique retaining analyte molecules on the column based on coulombic (ionic) interactions was developed (Clarke et al., 1999; Guignard et al., 2005; Zhang et al., 2012). Ion-exchange

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¹Corresponding author: shujuanyu8@gmail.com

chromatography can be used for almost any type of charged molecule, including reducing sugars and amino acids. In this experiment, the target analytes, including methylimidazoles, HMF, and reducing sugars (acid dissociation constant, pK_a , values of the imidazole ring and HMF were 7.70 and 12.82, respectively) were relatively transformed into anionic forms in alkaline solution. Thus, the simultaneous analysis of 4-MeI, 2-MeI, reducing sugars, and HMF by ion exchange chromatography should be an option. However, to the best of our knowledge, the simultaneous analysis of 4-MeI, 2-MeI, reducing sugars, and HMF is rarely reported in the literature.

Reducing sugars, 4-MeI, 2-MeI, and HMF were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Sodium hydroxide (50%) was of HPLC grade and purchased from Merck (Darmstadt, Germany).

The samples were filtered through a Millex-HN nylon clarification kit of 0.45- μ m pore size (Millipore, Bedford, MA), and analyzed on a DX 5000 Dionex system (Dionex Corp., Sunnyvale, CA), with a gradient pump (model EG40) with on-line degassing, and an integrated pulsed amperometric electrochemical detector (PAD, model ED40, Dionex Corp.). Separation was accomplished on an amino trap column (4 $\times$ 50 mm) coupled with a CarboPac PA1 analytical column (2 $\times$ 250 mm; Dionex Corp.) using 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 20 μ L. The pH reference electrode (061879, Dionex Corp.) and gold working electrode (061875, Dionex Corp.) were used in amino trap column with pulsed integrated amperometric electrochemical detector 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), where E_n is the applied voltage on work electrode, and t_n is the duration of the applied voltage. Integration occurred from 0.20 to 0.44 s during E_3 application.

The pH value of eluent was an important factor in dissociation of 4-MeI and 2-MeI. At pH values greater than the pK_a of the amino group (about 14.83), nitrogen was predominantly protonated as a negative charged imino group. As shown in Figure 1, 4-MeI and 2-MeI were separated on a CarboPac PA1 anion exchange column. The peaks of 4-MeI and 2-MeI were not symmetrical and had a long tail at low concentrations of eluent (e.g., 25 or 75 mmol/L NaOH). However, the peaks of 4-MeI and 2-MeI were symmetrical and narrow at 100 mmol/L NaOH. One proposed explanation was that 4-MeI and 2-MeI may be fully transformed

Figure 1. The separation of methylimidazoles (MeI) on a CarboPac PA1 anion exchange column (Dionex Corp., Sunnyvale, CA) without amino trap column affected by the concentration of NaOH. The y-axis indicates the signal in nanocoulombs (nC); peaks 1 and 2 represent 2-MeI and 4-MeI, respectively.

into the anionic forms at high concentrations of alkaline solution.

Glucose, mannose, maltose, 2-MeI, 4-MeI, and HMF always have different charges and could be retained by anion exchange column. As shown in Figure 2, glucose, mannose, maltose, 2-MeI, 4-MeI, and HMF could be detected by PAD. However, it was difficult to separate glucose, mannose, and HMF on a CarboPac PA1 anion exchange column. Meanwhile, 2-MeI and 4-MeI (retention time <8 min) were weakly retained on the CarboPac PA1 anion exchange column. Therefore, the CarboPac PA1 anion exchange column was not suitable for simultaneous detection of glucose, mannose, maltose, 2-MeI, 4-MeI, and HMF.

When a CarboPac PA1 anion exchange column-PAD combined with an amino trap column was used to improve separation and determination, glucose, mannose, maltose, 2-MeI, 4-MeI, and HMF could be fully separated and detected simultaneously (Figure 3). Documentation from Dionex (Document No. 031197-05) stated that the amino trap column was packed with 10- μ m, high-capacity anion exchange resin that had high selectivity for hydrophobic amino compounds relative to monosaccharides, using hydroxide eluents. Therefore, the retention of 2-MeI, 4-MeI, and HMF (retention times were 11.5, 20.8, and 49.5 min, respectively) were greatly improved by the amino trap column. However, the retention times of reducing sugars were changed slightly. Meanwhile, the peak shape of all target analytes was symmetrical and narrow. Thus, the CarboPac PA1 anion exchange column-PAD with

Figure 2. Analysis of methylimidazoles (MeI), reducing sugars, and 5-hydroxymethyl-2-furaldehyde (HMF) by high-performance anion exchange chromatography-electrochemical detector (CarboPac PA1 anion exchange column; Dionex Corp., Sunnyvale, CA) without amino trap column. The y-axis indicates the signal in nanocoulombs (nC); peaks 1, 2, 3, 4, 5, and 6 represent 2-MeI, 4-MeI, HMF, glucose, mannose, and maltose, respectively.

the amino trap column was suitable for simultaneous detection of glucose, mannose, maltose, 2-MeI, 4-MeI, and HMF.

Calibration curves were generated from calibration standards (HMF and maltose ranging from 1 to 100 mg/L; methylimidazoles, glucose, and mannose ranging from 0.1 to 10 mg/L). As shown in Table 1, calibration equations of all target analytes had excellent linearity. On the other hand, the limit of quantification (LOQ) was determined as 10 times the observed signal-to-noise ratio. In this experiment, as shown in Figure 3, the LOQ of glucose, mannose, maltose, HMF, 4-MeI, and 2-MeI were 0.02, 0.02, 0.10, 0.10, 0.02, and 0.02 mg/L, respectively. These results indicate that HPAEC-PAD coupled with an amino trap column is suitable for

Figure 3. Analysis of methylimidazoles (MeI), reducing sugars, and 5-hydroxymethyl-2-furaldehyde (HMF) by high-performance anion exchange chromatography-electrochemical detector (CarboPac PA1 anion exchange column; Dionex Corp., Sunnyvale, CA) with amino trap column. X represents the concentration of standard sample (1.25 mg/L for methylimidazoles, glucose, and mannose; 5 mg/L for HMF and maltose). The y-axis indicates the signal in nanocoulombs (nC); peaks 1, 2, 3, 4, 5 and 6 represent glucose, mannose, 2-MeI, 4-MeI, maltose, and HMF, respectively.

simultaneous analysis of reducing sugars, methylimidazoles, and HMF at trace concentrations.

In conclusion, an amino trap column improved the separation of methylimidazoles, reducing sugars, and HMF performed using an HPAEC-PAD system with a CarboPac PA1 anionic exchange column. The limit of detection for methylimidazoles and reducing sugars was 0.02 mg/L, whereas that for maltose and HMF was 0.1 mg/L using HPAEC-PAD. Therefore, simultaneous analysis of reducing sugars, methylimidazoles, and HMF at trace concentration by ion exchange chromatography is a valid and inexpensive choice for a small laboratory.

Table 1. Linear calibration ranges and regression equations of analytes as measured by amino trap column with pulsed integrated amperometric electrochemical detector (AMTC-PAD)

Analyte ¹	Calibration range (mg/L)	Regression equation ²	R ²	RSD ³ (%) (n = 6)
HMF	1.0–100.0	$y = 2.493x + 3.891$	0.998	2.10
4-MeI	0.1–10.0	$y = 16.480x + 0.479$	0.997	1.27
2-MeI	0.1–10.0	$y = 9.354x + 0.470$	0.999	1.73
Glucose	0.1–100.0	$y = 27.26x - 0.112$	0.998	2.34
Mannose	0.1–100.0	$y = 19.45x + 0.062$	0.998	3.15
Maltose	1.0–100.0	$y = 10.97x - 0.809$	0.998	2.16

¹HMF = 5-hydroxymethylfurfural; 4-MeI = 4-methylimidazole; 2-MeI = 2-methylimidazole.

²Where y = peak area of analytes (nC × min), where nC = nanocoulomb, and x = concentration of analytes (mg/L).

³Relative standard deviation.

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