Short communication: Simultaneous analysis of reducing sugars and 5-hydroxymethyl-2-furaldehyde at a low concentration by high performance anion exchange chromatography with electrochemical detector, compared with HPLC with refractive index detector

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ABSTRACT

A simultaneous analysis of reducing sugars and 5-hydroxymethyl-2-furaldehyde of the Maillard reaction products was detailed. It was based on a high performance anion exchange chromatography with electrochemical detector system and an HPLC with refractive index detector. Results showed that high performance anion exchange chromatography with electrochemical detector using a CarboPac PA-1 column (Dionex Corp., Sunnyvale, CA) was more suitable for reducing sugars and 5-hydroxymethyl-2-furaldehyde determination, especially for trace analysis. The lowest detectable limit of reducing sugars and 5-hydroxymethyl-2-furaldehyde was 0.00005 mol/L in this experiment. However, HPLC with a refractive index detector always produces a tailing peak for 5-hydroxymethyl-2-furaldehyde, and mannose and fructose cannot be absolutely separated. The results of the present study could provide a more sensitive means for 5-hydroxymethyl-2-furaldehyde and reducing sugar detection.

Key words: high performance anion exchange chromatography with electrochemical detector, HPLC with refractive index detector, 5-hydroxymethyl-2-furaldehyde, reducing sugar

Short Communication

The Maillard reaction is one of the most important and complex processes in food chemistry and processing (Moreno et al., 2003). Generally, the Maillard reaction occurs between the carbonyl group of a reducing sugar such as glucose and an amino compound, which then cyclizes to the N-substituted glycosylamine, and then forms the Amadori rearrangement product (ARP). One of the important characteristics of ARP is its ten-

dency to undergo enolization. Degradation of the ARP by 1,2-enolization and to form 3-deoxy-2-hexosulose has been confirmed as a main factor to produce 5-hydroxymethyl-2-furaldehyde (HMF; Hodge, 1953; Davidek et al., 2002; Moreno et al., 2003; Rufián-Henares et al., 2006; Rufián-Henares and Morales, 2007; Guan et al., 2012).

Dairy thermal treatment always produces HMF (its safety for humans uncertain) via the Maillard reaction. Some of the early studies suggested that HMF has potential toxic, mutagenic, and carcinogenic effects (Nässberger, 1990; Michail et al., 2007). A high concentration of HMF always means low quality of the dairy product. Many studies have detailed the detection of HMF using modern instruments. One of the most frequently used methods for analyzing reducing sugars and HMF is based on HPLC with various types of chromatographic columns and detectors (Davidek et al., 2005). Morales and Arnoldi (1999) performed the HPLC separation of HMF from a lactose-caseinate reaction system. Drusch et al. (1999) used a modified reversed-phase-HPLC method with o-phthalaldehyde precolumn derivatization for determination of precursors of HMF in food samples. Guan et al. (2011) used HPLC with a refractive index detector (HPLC-RID) system to analyze reducing sugars. However, HPLC techniques cannot simultaneously separate and detect reducing sugars and HMF simultaneously and are not convenient for postcolumn derivatization operation (Davidek et al., 2005).

Recently, means based on high performance anion exchange chromatography (HPAEC) coupled with an electrochemical pulsed amperometric detection or diode array detector have been reported as a powerful analytical technique for the detection and monitoring of known traces in food material (Davidek et al., 2002, 2005; Joo et al., 2008). These methods could simultaneously analyze reaction precursors and products. However, almost no research concerning the simultaneous analysis of HMF and reducing sugars has been

Received April 15, 2012.

Accepted July 21, 2012.

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Table 1. Gradient program for the high performance anion exchange chromatography with electrochemical detector (HPAEC-ECD) analysis of Maillard reaction product and reducing sugars

Retention (min)	Flow (mL/min)	Gradient (% by vol)		
		Water	NaOH (500 mM)	NaOAc ¹ (500 mM)
-10	1	97	3	0
0	1	97	3	0
30	1	95	3	2
35	1	49	3	48
40	1	49	3	48

¹Sodium acetate.

reported in recent literature. The aim of this work was to develop simultaneous determination of reducing sugars and HMF using an HPAEC with electrochemical detector (HPAEC-ECD) system and to compare it with the HPLC-RID method.

D-Glucose, D-fructose, and D-mannose were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). 5-Hydroxymethyl-2-furaldehyde was purchased from Sigma-Aldrich (St. Louis, MO).

The mixed solution of D-glucose (0.1 mmol/mL), D-fructose (0.1 mmol/mL), D-mannose (0.1 mmol/mL), and HMF (0.1 mmol/mL) was analyzed using an HPLC-RID system. Samples were filtered through a Millex-HN nylon clarification kit of 0.45- μ m pore size (Millipore Corp., Bedford, MA) and then analyzed using an HPLC system. The HPLC system configuration

was as follows: chromatogram controller: Waters 600 (Waters Corp., Milford, MA), pump: Waters 600E (Waters Corp.), injector: Rheodyne 7725i manual injector (Waters Corp.), and detector: Waters 2414 refractive index detector (Waters Corp.). The HPLC-RID analysis using Sugar-pak1 6.5 $\times$ 300 mm ion-exchange chromatography (Waters Corp.) was performed as follows: the injection volume was 5 μ L and the mobile phase was a 50-mg/L EDTA-Ca water solution delivered at a flow rate of 0.5 mL/min. The column temperature was set at 90°C. The chromatography running time was 30 min. The HPLC peaks were identified to be D-glucose, D-fructose, D-mannose, and HMF by comparing the retention time between them and standard compounds.

The HPAEC-ECD analysis was done according to the literature, with a slight modification (Davidek et

Figure 1. High performance liquid chromatography with refractive index detector (HPLC-RID) analysis of reducing sugars and 5-hydroxymethyl-2-furaldehyde (HMF).

al., 2003). The mixed solution of D-glucose (0.1 mmol/mL), D-fructose (0.1 mmol/mL), D-mannose (0.1 mmol/mL), and HMF (0.1 mmol/mL) were filtered through a Millex-HN nylon clarification kit of 0.45- μ m pore size (Millipore Corp.), and then analyzed by a DX 5000 Dionex system (Dionex Corp., Sunnyvale, CA), a gradient pump (model EG40) with online degassing, and an electrochemical detector (model ED40). Separation was accomplished by a CarboPac PA1 anion exchange column (250 $\times$ 4 mm; Dionex Corp.) and a CarboPac PA1 guard column (50 $\times$ 4 mm; Dionex Corp.) with a constant flow rate of 1 mL/min throughout the program (the gradient is shown in Table 1). The injection volume was 10 μ L.

D-Glucose, D-fructose, D-mannose, and HMF always have different charges and could be reserved by cation or anion stationary phases. As shown in Figure 1, HMF, D-glucose, D-fructose, and D-mannose could be detected by RID. However, it was difficult to separate mannose and fructose, as the reducing sugars concentration was 1 mol/L. Even at a concentration of 0.1 mol/L, mannose and fructose could not be absolutely separated. Moreover, HMF produced a tailing peak, as the concentration was 0.1 and 1 mol/L, and almost

no peak was observed, as the concentration was 0.001 mol/L or lower than 0.001 mol/L, which means that the lowest detectable limit (**LDL**) using the HPLC-RID system for reducing sugars and HMF simultaneous detection was no lower than 0.001 mol/L in this experiment. Therefore, HPLC-RID was not suitable for reducing sugars and HMF simultaneous detection at a low concentration.

As the HPAEC-ECD with a CarboPac PA1 anion exchange column was used for separation and determination, D-glucose, D-fructose, D-mannose, and HMF could be separated and detected simultaneously when the concentration was from 0.00005 to 0.01 mol/L (Figure 2). The standard lines of HMF and reducing sugars are shown in Figure 3. However, mannose and fructose could not be completely separated if the concentration was 0.01 mol/L or higher than 0.01 mol/L. The reason for this was that CarboPac PA1 anion exchange column was overloaded when the reducing sugar and HMF injection concentration was 0.01 mol/L or higher than 0.01 mol/L. On the other hand, almost no peak of reducing sugars and HMF could be observed when the concentration was 0.00001 mol/L, whereas both reducing sugars and HMF peaks were observed when

Figure 2. High performance anion exchange chromatography with electrochemical detector (HPAEC-ECD) analysis of reducing sugars and 5-hydroxymethyl-2-furaldehyde (HMF). Color version available in the online PDF.

Figure 3. Standard lines of reducing sugars and 5-hydroxymethyl-2-furaldehyde (HMF) using high performance anion exchange chromatography with electrochemical detector (HPAEC-ECD) analysis. Color version available in the online PDF.

the concentration was 0.00005 mol/L, which means that the LDL using HPAEC-ECD to detect D-glucose, D-fructose, D-mannose, and HMF was 0.00005 mol/L in this experiment. Thus, it can be seen that HPAEC-ECD is more suitable for trace analysis of reducing sugars and HMF.

The HPAEC-ECD system with a CarboPac PA1 anion exchange column was more suitable for separating and detecting reducing sugars and HMF than HPLC-RID at a trace concentration. The LDL of reducing sugars and HMF was 0.00005 mol/L using HPAEC-ECD.

ACKNOWLEDGMENTS

All authors acknowledge the National Science Foundation of China (Beijing, China; grant no. 31071564), the Science Foundation of Guangdong Province of China (grant no.10251064101000014), and the Fundamental Research Funds for the Central Universities (China; 2011ZP009) for financial support.

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