J. Dairy Sci. 95:6379–6383
http://dx.doi.org/10.3168/jds.2012-5623

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

Y.-G. Guan,* P. Yu,* S.-J. Yu,*†[1] X.-B. Xu,* and X.-L. Wu*
*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 510631, China

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 tendency to undergo enolization. Degradation of the ARP by 1,2-enolizationand 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.
[1]Corresponding author: shujuanyu8@gmail.com

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

		Gradient (% by vol)		
Retention (min)	Flow (mL/min)	Water	NaOH (500 m*M*)	NaOAc[1] (500 m*M*)
−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

[1]Sodium 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-μm 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 × 300 mm ion-exchange chromatography (Waters Corp.) was preformed as follows: the injection volume was 5 μL 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

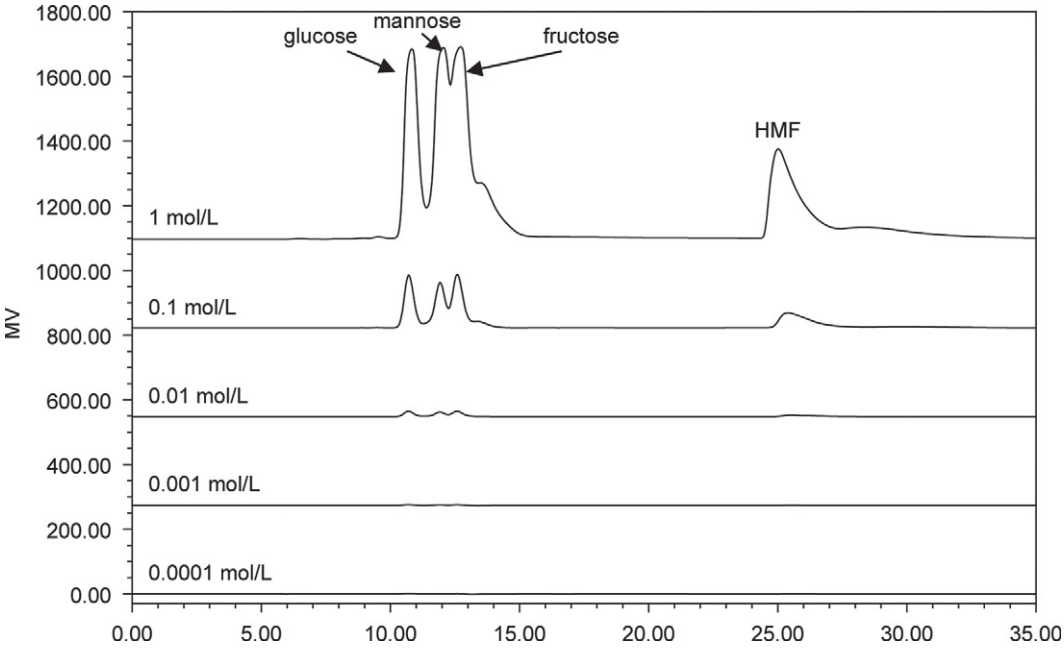

Figure 1. High performance liquid chromatography with refractive index detector (HPLC-RID) analysis of reducing sugars and 5-hydroxymethyl-2-furaldehyde (HMF).