

Liquid Chromatography for the Determination of 5-(Hydroxymethyl)-2-furaldehyde in Breakfast Cereals

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A simple procedure for the determination of (hydroxymethyl)furfural (HMF) in breakfast cereals is reported. HMF was extracted with solvent, and separation was performed on a reversed-phase C₁₈ column with water-acetonitrile (95:5 v/v). UV detection was set at 285 nm. Average recovery of HMF was 97.7%, and the detection limit for the samples investigated was 0.03 µg/g. The concentrations of HMF in breakfast cereals ranged from 3.7 to 193.3 µg/g.

INTRODUCTION

Heat treatment of foods rich in reducing sugars may result in Maillard or nonenzymatic browning. This reaction occurs between reducing sugars and amino acids, peptides, or proteins, causing a change in the color, flavor, functional properties, and nutritional value of the food (O'Brien and Morrissey, 1989).

During extrusion the Maillard reaction is favored by conditions of high temperature (>180 °C) and shear (>100 rpm) in combination with low moisture (<15%) (Cheftel, 1986). Starch and nonreducing sugars such as sucrose may be hydrolyzed to form reducing sugars which favor nonenzymatic browning. This reaction is more likely to occur in expanded snack food rather than in breakfast cereals and other foods with higher moisture contents (Camire et al., 1990).

Many methods are available for evaluating the extent of Maillard reaction in foods. Among these are chemical methods [measurements of "available" lysine, furosine, ε-pyrrole-lysine and (hydroxymethyl)furfural (HMF)], immunoassays, mammalian growth bioassays, and microbiological assays. Although HMF is produced in food through the dehydration of sugars, the determination of HMF is still a useful, simple, and widely employed technique for evaluating the extent of the Maillard reaction. The classic colorimetric methods (Winkler, 1955; Keeney and Bassette, 1959) are being replaced by chromatographic techniques, which offer greater specificity and more precision. High-resolution liquid chromatography is presently the most common technique, having been used to determine HMF in such products as juices and fruit concentrates (Porretta and Sandei, 1991; Li et al., 1989), milk (Van Boekel and Rehman, 1987; Fernandez et al., 1992), coffee (Dauberte et al., 1990), beer (Morawski et al., 1983), wine (Williams et al., 1983), honey (Jeuring and Kupperts, 1980), and baby cereals (Guerra-Hernández et al., 1992).

In this paper we propose a simple procedure for HMF determination, useful for evaluating the extent of Maillard reaction in breakfast cereals. This method may be used to control the heating procedure of those products as well as other cereal derivatives.

EXPERIMENTAL PROCEDURES

Apparatus. The liquid chromatographic system used in this study consisted of a Perkin-Elmer with a 20-µL injection loop chromatograph, a Spherisorb S5 ODS2 (250 mm × 4 mm i.d.) column, and a UV detector Model 235 set at 285 nm. The mobile phase was water-acetonitrile (95:5).

Reagents. Analytical reagent grade chemicals were used. The clarified solution was composed of potassium ferrocyanide 15% (w/p) (Merck) (Carrez I) and zinc acetate 30% (w/p) (Merck) (Carrez II).

A standard stock solution containing 200 mg/L 5-(hydroxymethyl)furfural (Merck) was used to prepare the working standard solutions (0.05–0.5 and 0.5–5.0 mg/L).

Samples. Twenty-three samples of breakfast cereals were obtained from six brands commercialized in Spain.

Procedure. Ground sample (0.4 g) was weighed into a 10-mL centrifuge tube to which 7 mL of deionized water was then added. The centrifuge tube was shaken vigorously for 1 min, and the sample was then centrifuged for 10 min at 5000 rpm. The same procedure was followed twice more. The supernatants were clarified with 1 mL each of Carrez I and II solutions. The resulting mixture was centrifuged for 5 min at 5000 rpm. The solution was diluted to a total volume of 25 mL with deionized water. One aliquot of 2 mL was filtered, before injection, through a 0.45-µm disk filter.

Chromatographic Conditions. Twenty microliters of the filtered solution was separated on a reversed-phase C₁₈ column with a flow rate of 1 mL/min. The HMF was completely separated out in 6 min.

The Sigma Package (Horus Hardware) was used to obtain the linear regression equations. The HMF dissolution concentrations and the height of the peak obtained were considered as the variables for these equations. The concentration ranges were 0.05–0.5 and 0.5–5.0 mg/L, with correlation coefficients of 0.999 for both curves. The linear regression equations used were ($n = 6$) $Y = 2.91 + 162.94X$ and $Y = 0.42 + 32.39X$, respectively, where Y is the peak height and X is the HMF concentration.

The HMF sample solution concentration was obtained from the calibration curves of the standard solution.

RESULTS AND DISCUSSION

The external standard method was used for HMF determination. Typical chromatograms of a standard solution of HMF and a cornflake sample chromatogram (brand A1) are shown in Figure 1.

The accuracy of the method was tested with recovery assays in the cereal samples with lower concentrations of HMF (3.67 µg/g). Increasing quantities of a standard solution of HMF were added to the sample, and recovery was carried out as described above (see Table I for results). The values correspond to an average of five samples for each concentration added and to at least three injections per sample. The recovery range was 95.65–100.00%, and the average value was 97.73%. The greatest accuracy obtained was for concentration values between 10 and 50 µg/g.

The detection limit was 0.03 µg/g (signal-to-noise ratio of 2). The minimum HMF concentration found in the

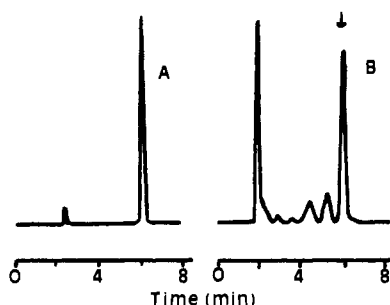


Figure 1. Typical chromatograms of HMF standard solution (A) and cornflake sample (B). The arrow indicates the HMF peak in the chromatogram.

Table I. HMF Recovery in the Analysis of Breakfast Cereal Samples^a (Micrograms per Gram)

added	total	detected	recovered, %
10	13.67	13.62	99.63
25	28.67	28.67	100.00
50	53.67	53.08	98.90
75	78.67	75.92	96.50
100	103.67	99.20	95.69
150	153.67	146.98	95.65
			av 97.73 ± 1.83

^a Cereal contained 3.67 µg/g HMF.

Table II. Precision Study

sample	HMF content, ^a µg/g	RSD, %
C3	3.67 ± 0.120	3.27
A1	52.97 ± 0.786	1.48
D2	98.16 ± 1.175	1.19

^a Mean standard deviation based on 12 replicate analyses.

Table III. HMF Content of Breakfast Cereals Analyzed

sample	type of cereal	HMF, µg/g	sample	type of cereal	HMF, µg/g
brand A			brand C		
1	corn	52.97	1	corn, wheat, oats	13.75
2	wheat	61.00	2	oats, wheat	5.10
3	wheat	20.73	3	rice, wheat	3.67
4	rice, wheat	14.23	brand D		
5	oats, wheat	6.04	1	rice	6.82
6	corn	55.02	2	wheat	4.85
7	rice	9.62	3	corn	3.70
8	corn	134.37	brand E		
9	corn	20.50	1	corn	14.89
brand B			2	wheat	193.34
1	rice	16.13	brand F		
2	corn	12.05	1	wheat, rye	16.87
3	corn	26.04	2	wheat, corn	23.77
4	wheat	98.16			

samples analyzed exceeded the analytical detection limit by approximately 100 times.

The method was optimally precise. Twelve determinations on three different types of cereals were used for this assay, the results of which are summarized in Table II. The concentration ranges of the cereals tested cover most of the HMF values found in breakfast cereals.

We have analyzed 23 breakfast cereal samples from 6 brands commercialized in Spain. Table III lists the mean HMF content found in the breakfast cereals. The con-

centration range was between 3.67 and 193.34 µg/g, although 70% of the samples had HMF concentrations lower than 25 µg/g and only two samples had values higher than 100 µg/g. There was no relationship between the type or brand of cereal analyzed and the HMF content.

The method proposed is suitable for evaluating the extent of Maillard reaction in breakfast cereals. No references have been found in bibliography consulted on methods of HMF determination in breakfast cereals.

The method tested is simple and rapid; HMF separation time was 35 min at 5000 rpm, and HMF detection by HPLC required 6 min. It showed excellent results in both precision and recovery.

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