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DETERMINATION OF HYDROXYMETHYLFURFURAL IN BABY CEREALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A liquid chromatography method for the detection and quantification of 5-hydroxymethyl-2-furaldehyde (HMF) in baby cereals is described. HMF was obtained by centrifugation and the supernatant was clarified by the addition of Carrez solution. The mixture was centrifuged for 5 min at 5,000 rpm. The samples were analysed on a reverse-phase C_{18} column and ultraviolet detector (284 nm) in isocratic elution with 95:5 water-acetonitrile. Average recovery of HMF by the standard addition method was 96.9%. The variation coefficients for three levels of concentration, (3.24, 22.82 and 65.50 ppm) were 2.40, 2.14 and 1.09% respectively. Limit of detection was ≤ 0.09 ppm. This method was applied by determination of HMF in commercial baby cereals.

INTRODUCTION

The Maillard reaction may seriously reduce the nutritional value of foods through the destruction of essential amino acids and the production of antinutritive and toxic compounds.

Nutritionally, the destruction of lysine is the most significant consequence and is of greatest importance in those foods in which this amino acid is limited, such as in cereals (1).

There is also evidence of inhibition of enzymatic digestive activity produced by certain compounds in Maillard reaction (2,3). Other factors of interest include the effect of this reaction on the metabolism and the bioavailability of minerals (4) and the allergenicity of food proteins, especially in children (5). The Maillard reaction can be kept track of by HMF determination. Methods of HMF analysis developed up to now, however, are based on colorimetric reactions which are neither specific nor very precise (6,7). Chromatographic techniques, on the other hand, offer greater specificity in the determination of this compound. High-resolution liquid chromatography is the most common technique presently, having been used to determine HMF in such products as juices and fruit concentrates (8,9), milk (10), coffee (11), beer (12), wine (13), and honey (14).

The object of this experiment was to perfect a method for the rapid extraction and determination of HMF in baby cereals using HPLC, the purpose being to control the heating process of these products.

MATERIALS

Reagents

a) **Mobile phase.**- Acetonitrile-water mixed solvent (5:95).

Acetonitrile, HPLC grade (E. Merck, D-6100 Darmstadt, Germany).

b) **Carrez solution I.**- Potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) (Merck) in water 15% (w/v).

c) **Carrez Solution II.**- Zinc acetate ($Zn(CH_3CO_2)_2 \cdot 2H_2O$) (Merck) in water 30% (w/v).

d) 5-(Hydroxymethyl)-furfural standard solutions.-

5-(Hydroxymethyl)-furfural (HMF) (E. Merck, D-6100 Darmstadt, Germany).

Stock solutions.- 200 mg/L. Prepared by dissolving 0.02 g in water and diluting to 100 mL.

Working standard solutions I.- Diluted stock solution in water to 10 mg/L.

Working standard solutions II.- Diluted stock solution in water to 1 mg/L.

High-purity certified reagents were used for all analyses.

Apparatus

e) **Liquid chromatography.-** Model 500-A with 20 μ l injection loop, a Model UV-200 spectrophotometer, both from Konic (Instruments. S.A., Barcelona, Spain) and a model 3394-A integrator from Hewlett-Packard (Santa Clara, CA).

g) **Chromatographic column.-** Spherisorb S5 ODS2, 250mmx4mm I.D.(Deeside Ind.Est. Queensferry,Clwdy.UK).

Samples

Twelve samples of baby cereals from the two most popular brands in Spain have been analyzed (for their composition see Table 2).

METHOD**Sample preparation**

2 g of baby cereal (weighed to 0.1 mg) was added to a 100 mL centrifuge tube with 25 mL of de-ionized water and was mixed for 2 min. The samples were then centrifuged for 10 min at 5,000 rpm, after which the supernatant was separated out. Another 25 mL of de-ionized water was added

to the sample and the same procedure was followed once again. The two supernatans were then mixed together and a 25 mL aliquot was clarified with 0.5 mL each of Carrez I and II solutions. The resulting mixture was then centrifuged for 5 min. at 5,000 rpm.

2 mL of clear supernatant was pipetted into a Hamilton syringe and filtered, before injection, through a 0.45 μm disc filter.

Liquid Chromatography

With mobile-phase flow rate of 1 mL/min and a sensitivity of 0.005 and 0.2 AUFS for different concentration ranges, 20 μL of sample was injected. The detector wavelength was 284 nm and all separations were performed at room temperature.

RESULTS

The external-standard method was used for the HMF determination by HPLC. Two calibration curves were obtained by plotting the mean peak heights vs HMF concentration. The concentration ranges were 0.01 at 0.4 mg/L and 0.2 at 3.0 mg/L. The correlation coefficients were 0.999 for both curves.

Figure 1 shows a typical chromatogram of a standard solution of HMF as well as a chromatogram of a sample (sample F) using the proposed technique.

Precision

The precision of the method was measured in three types of samples with concentration ranges of 3.25, 22.82 and 65.5 ppm, these cover the HMF values found in the cereals analyzed.

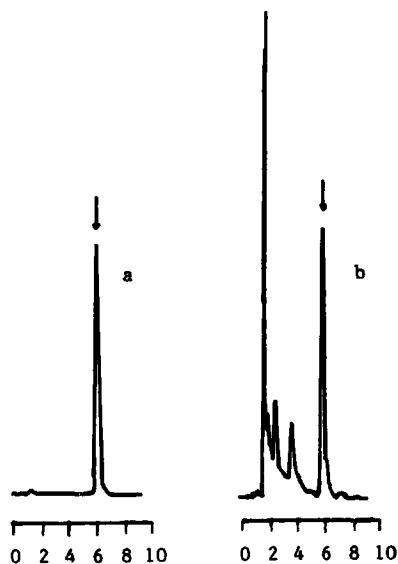


Figure 1.- Chromatograms of an HMF Standard Solution (a) and Baby Cereal Sample (b). The Arrow Indicates the HMF Peak in the Chromatogram.

The samples were analyzed 11 times. The variation coefficients were 2.40%, 2.14% and 1.09% for concentration levels of 3.25, 22.82 and 65.5 ppm respectively.

Limit of detection

The lower limit of determination in the sample with a signal-to-noise ratio $\leq 2:1$ is 0.09 ppm by this method.

Accuracy

Recovery was carried out on a sample of baby cereal (Sample F) with 22.8 ppm of HMF (average of 11 determinations). Increasing quantities of a

TABLE 1
Recovery of Hydroxymethylfurfural Added to
Cereal Sample* (ppm).

ADDED (ppm)	TOTAL (ppm)	FOUND** (ppm)	REC %
10	32.8	31.0	94.5
20	42.8	40.5	94.6
30	52.8	49.3	93.4
40	62.8	62.8	100.0
50	72.8	74.1	101.8
60	82.8	84.1	101.6
70	92.8	90.8	97.8
80	102.8	94.8	92.2

Av.: 96.9±3.6

(*) Cereal contained 22.8 ppm HMF.

(**) Mean of 3 runs with at least 3 injections
for each run.

standard solution of HMF were added to the sample, and recovery proceeded as described in the method.

Concentrations of added and recovered HMF are given in Table 1. The values correspond to an average out of 3 samples for each added concentration and to at least 3 injections per sample. The average recovery over the concentration range 32-103 ppm HMF was 96.5±3.6.

The method was used to analyze 12 commercial samples of baby cereals (hydrolyzed and toasted) from the two most popular brand names in Spain. Results given in Table 2 shown the concentrations of HMF in the different products as well as the type of cereals in each formula. The concentration range varied widely, from 3.25 ppm in the creamed rice to 65.5 ppm in formulas which include cereals and fruits.

TABLE 2
HMF Values in Different Commercial Baby Cereals

<u>BABY CEREAL</u>	<u>TYPE OF CEREAL</u>	<u>HMF(ppm)</u>
MANUFACTURER 1		
A*	wheat,soy,rice,oat,barley,rye	12.50
B	rice,maize,soy	10.07
C	rice	0.44
D	oat	5.38
E	wheat	39.50
F	wheat,maize,rice,oat,barley,soy, rye,millet	22.82
MANUFACTURER 2		
A*	wheat	10.32
B	rice,maize,soy,cornstarch	6.50
C	rice,maize,cornstarch,tapioca	4.82
D	rice	3.25
E*	wheat,rice,oat,barley,millet	11.65
F**	wheat,rice,barley	65.50

(*) Samples with honey.

(**) Sample with fruits.

TABLE 3
HMF Values in Different Sample
Amounts

<u>SAMPLE</u> <u>(grams)</u>	<u>HMF</u> <u>(ppm)</u>
0.5	23.4
1.0	22.8
1.5	23.1
2.0	22.8
2.5	22.1
3.0	22.6
Av.	22.8±0.40

DISCUSSION

We have proposed a method for the determination of HMF in baby cereals, having found no references in the bibliographies consulted about methods of HMF determination in this product.

HMF recovery averages was 97% using this method; however, two centrifugations are required since the first yields only 76% recovery. The greatest accuracy is obtained for concentration values between 60 and 80 ppm (Table 1).

Separation time is 25 min at 5,000 rpm.

Precision was optimum for all three concentration levels tested.

This method is applicable only to sample quantities from 0.5 to 3.0 g (Table 3), since for larger amounts it is necessary either to increase the amount of water added or the centrifugation speed.

A high chromatographic resolution was obtained with the mobile phase in 6 minutes for all types of samples analyzed.

This method may prove to be extremely valuable when used in order to control the effect of heat treatment cereals.

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