Furosine Determination in Baby Cereals by Ion-Pair Reversed-Phase Liquid Chromatography

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ABSTRACT

A simple procedure for the determination of ε-*N*-2-furoylmethyl-L-lysine (furosine) in baby cereals is reported. Furosine was quantified in acid hydrolysates by isocratic ion-pair reversed-phase liquid chromatography using a C_{18} column and UV detection at 280 nm. The detection limit was 1.42 pmol. Furosine formation by sample hydrolysis with hydrochloric acid increased with acid concentration to 10.6*M*. The content of furosine found in baby cereals ranged from 293 to 3,180 mg/100 g of protein.

Technological processes applied to food can give rise to modifications in their composition. One of the most important modifications induced in food by heating is the Maillard reaction, which involves amino acids and reducing carbohydrates, and can also produce loss of nutritive value (Henle et al 1991).The loss of available lysine is the most significant consequence of this reaction, and it is of the greatest importance in those foods where this amino acid is limiting, such as in cereals (O'Brien and Morrisey 1989) as well as other processed foods (Erbersdobler and Hupe 1991).

In infant formulas, the spray-drying process can promote the Maillard reaction. Because of the mild operating conditions in these products, the reaction is limited to its early stages, leading to the formation of the Amadori compounds (Evangelisti et al 1994).

Evaluation of the early stages of Maillard reaction, can be achieved by the determination of the furosine (ε-*N*-(furoylmethyl)-L-lysine) amino acid formed during acid hydrolysis of the Amadori compound fructosyl-lysine, lactulosyl-lysine, and maltulosyl-lysine produced by reaction of ε-amino groups of lysine with glucose, lactose, and maltose (Erbersdobler and Hupe, 1991).

The first reported methods for furosine determination using an ion-exchange amino acid analyzer may give an underestimation of lysine damage (Henle et al 1991). Gas chromatography has been used too but there are some problems with the derivatization (Ruttkat and Erbersdobler 1994). High-performance liquid chromatography (HPLC) methods remove these disadvantages and allow a shortened analysis time (Schleicher and Wieland 1981, Chiang 1983, Drexel et al 1987, Resmini et al 1990, Delgado et al 1992).

During the last few years, the furosine determination has been used for the evaluation of lysine modification in different foods (López-Fandiño et al 1993, Evangelisti et al 1994, Henle et al 1995), as well as in biological materials (Cefalu et al 1991). The furosine determination has been utilized also in cereals products to monitor the extent of nonenzymatic browning in cakes prepared with high-fructose corn syrup (Harris and Johnson 1987) and to control pasta product processing (Resmini and Pellegrino 1994).

Baby cereals are hydrolyzed during the processing and the reducing sugar level increases, thus toasting and drying processes can promote Maillard reaction to a major extent. However, no data are available on the furosine content in this type of food. In this article, we propose a simple ion-pair reversed-phase liquid chromatography RP-HPLC procedure for furosine determination in baby cereals to control processing conditions and to evaluate the early extent of Maillard reaction.

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MATERIALS AND METHODS

Apparatus

A Beckman System Gold chromatograph (Beckman Instruments, Fullerton, CA) consisting of a programmable solvent module (model 126) and a variable-wavelength UV detector, was used. Data were collected by a System Gold Software data system (Beckman Instruments).

Reagents

Analytical reagent-grade chemicals were used. The furosine standard was obtained by acid hydrolysis of ε-*N*-(1-deoxy-D-fructosyl)-L-lysine according to the procedure of Finot et al (1968). A standard stock solution containing 1.2 mg/100 ml of furosine was used to prepare the working standard solution.

Samples

Ten commercial baby cereals from the two most popular brands in Spain were examined. Samples were labeled as hydrolyzed from starch; the samples from Manufacturer 2 were also toasted. All were purchased in a local pharmacy and their composition is reported in Table I.

Sample Preparation

Furosine was determined after hydrolyzing 150 mg of sample, weighed with analytical accuracy, with 4.5 ml of 10.6*M* HCl at 110°C for 24 hr in a Pyrex screw-cap vial with PTFE-faced septa. High-purity helium gas was bubbled through the solution for 2 min. Sample preparation for HPLC analysis followed the method of Resmini et al (1990). The hydrolysate was filtered with a medium-grade paper filter. A 0.5 ml portion of the filtrate was applied to a Sep-pak C_{18} cartridge (Millipore) prewetted with 5 ml of methanol and 10 ml of water, then eluted with 3 ml of 3*M* HCl and evaporated under vacuum. The dried sample was dissolved in 3 ml of a mixture of water, acetonitrile, and formic acid (95:5:0.2) before HPLC analysis. Duplicate sample hydrolysates were used.

Chromatographic Conditions

The furosine was quantified by ion-pair reversed-phase HPLC according to the method of Delgado et al (1992), using a Spherisorb ODS2 5-μm column (250 × 4.6 mm, i.d.; Phenomenex, Torrance) operating at ambient temperature. The mobile phase consisted of a solution of 5 m*M* sodium heptane sulphonate with 20%

acetonitrile and 0.2% formic acid. The elution was isocratic and the flow rate was 1.2 ml/min. The UV detector was set at 280 nm and injection volume was 50 µl. Calibration of the chromatographic system for furosine determination was made by the external standard method.

RESULTS AND DISCUSSION

Typical chromatograms of a standard solution of furosine and of a baby cereal sample are shown in Figure 1. It can be observed that furosine was completely separated out in 8 min. In all cereal samples studied, no interfering peaks were present at furosine retention time.

Calibration was performed by adding increasing quantities of furosine standard within the expected concentration range to a previously hydrolyzed wheat flour sample. A calibration curve was constructed by plotting the measured absorbance, expressed in units of area versus micrograms of injected furosine. The equation for the curve was:

$$y = 33.415584 \times + 0.09063$$

A linear response was confirmed in the studied range (1.02×10^{-3} – 1.02 µg injected) with a high correlation coefficient (r^2 = 0.999604). The detection limit was 1.42 pmol (calculated as three times the standard error).

The precision of the entire assay procedure including acid hydrolysis, sample preparation, and RP-HPLC analysis (same day) was evaluated on a sample containing rice and maize (n = 7). The relative standard deviation (RSD) was 3.73% obtained on a sample with an average furosine value of 2,050 mg/100 g of protein.

Reproducibility of the chromatographic method was determined by injecting the same acid hydrolysate seven times for seven days. A RSD of 1% was obtained from a sample with an average furosine value of 1,930 mg/100 g of protein.

Recovery (Table II) was obtained by using an acid hydrolysate of toasted wheat sample, to which increasing amounts of furosine standard were added. The concentration range studied was 27.85–3,480.12 mg of furosine/100 g of protein. The recovery range was 90.5–98.1%, and the average value was 94.7. Similar recovery was obtained by Resmini et al (1990). The best recoveries were obtained for concentrations between 40.57 and 1,328.45 mg of furosine/100 g of protein.

The influence of the concentration range of 6N to 11.7N hydrochloric acid on furosine formation was studied. As observed in Figure 2, increasing concentrations of hydrochloric acid lead to significantly higher furosine values, except for the highest concentration, which may cause a degradation of the furosine. This is in agreement with the results obtained by other researchers in different foods (Molnár-Perl et al 1986, Henle et al 1995). Since no further increase of furosine amount was found with the highest concentration of hydrochloric acid, 10.6N HCl was used.

The method was used to analyze 10 commercial baby cereal samples from the two most popular brands in Spain. The amounts of furosine found in these samples as well as the ingredients of

TABLE I
Furosine Content in Commercial Baby Cereal

Samples[a]	Ingredients	Protein Content (%)	Furosine (mg/100 g of protein)
Manufacturer 1			
1	Wheat, rice, rye, fruits	7.66[b]	1,860
2	Rice	7.70[c]	2,840
3	Soy, rice, maize	11.04[d]	3,180
4	Rice, maize	6.07[d]	2,050
5	Wheat, rice, barley, rye, maize, oat, millet, soy, honey	7.74[b]	1,370
Manufacturer 2			
6	Wheat, rice, barley, rye, maize, millet, oat, fruits	7.17[b]	428
7	Soy, rice, maize, fruits	8.05[d]	1,010
8	Wheat, rice, barley, rye, maize, millet, oat, biscuit, orange, banana	8.08[b]	379
9	Wheat, rice, barley, rye, maize, millet, oat, sorghum, honey	11.89[b]	819
10	Wheat, rice, barley, rye, maize, millet, oat	10.65[b]	293

[a] n = 2.
[b] N × 5.70.
[c] N × 5.95.
[d] N × 6.25.

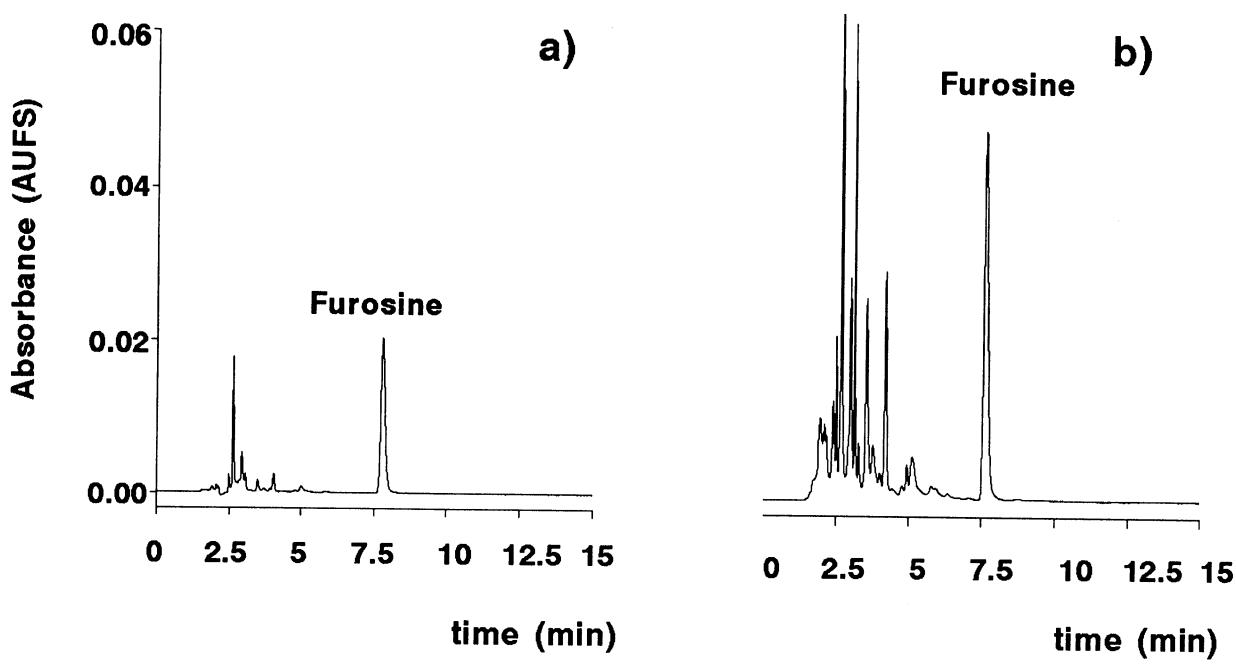

Fig. 1. High-performance liquid chromatography (HPLC) chromatograms of a furosine standard solution (a) and of a baby cereal sample (b).

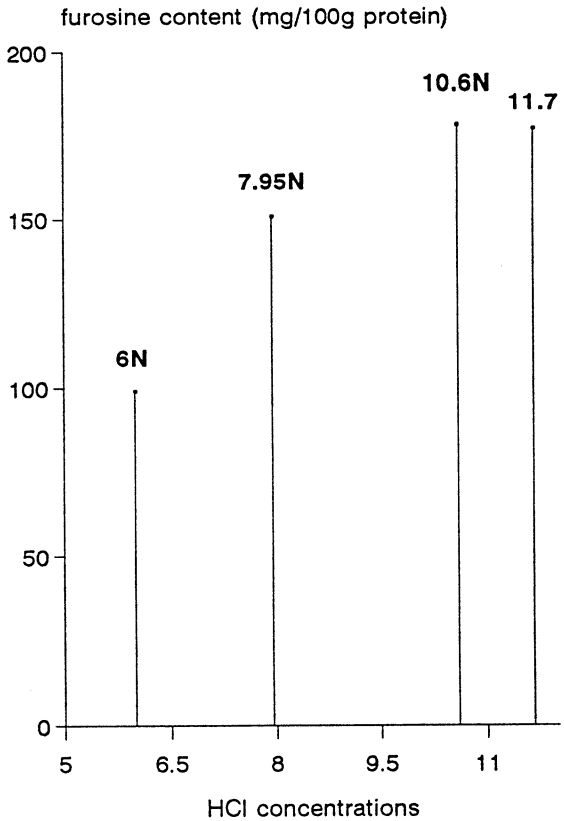

Fig. 2. Effect of different concentrations of hydrochloric acid on the formation of furosine in a wheat baby cereal sample.

each product are shown in Table I. Protein contents were also reported to provide more information about one of the principal components involved in the Maillard reaction. The furosine concentration ranges varied from 1,370 to 3,180 mg/100 g of protein and 293 to 1,010 mg/100 g of protein for samples of Manufacturers 1 and 2, respectively. The higher furosine values found in samples from Manufacturer 1 may be due to more severe processing conditions. Samples 3 (Manufacturer 1) and 7 (Manufacturer 2) labeled as "growth", showed the highest furosine values from the two manufacturers and contained soy as the major component. Samples 6 and 10, from the same manufacturer, with similar cereal composition, showed different furosine levels. This may be due to the presence of fruits in sample 6, which increases the amount of reducing monosaccharides involved in the Maillard reaction. This same could be observed in sample 9, but in this case the monosaccharide fraction was provided by honey.

Carratù et al (1993) found 50 and 120 mg of furosine /100 g of protein in two cereal flour samples for baby nutrition. The different level found in our samples can be attributed to different processing conditions, as well as to analytical methodology used. Because no other data were available in the bibliography consulted, we compared our results with samples that have been exposed to an additional drying step. Resmini et al (1990) found a wide range (319–4,682 mg/100 g of protein) of furosine in spray- and roller-dried milk. Henle et al (1995) obtained a high furosine content in powder infant formula in the range of 980–1,890 mg/100 g of protein.

Although baby cereal foods have less lysine content than dried milk and powder infant formula, the furosine level found was similar. This can be attributed to severe processing conditions. The method described in this article is suitable for evaluating the extent of the Maillard reaction in powder baby cereals.

TABLE II
Furosine (mg/100 g of protein)Recovery in the Analysis of Baby Cereal[a]

Added	Total	Detected	Recovered (%)
27.85	41.66	40.57	97.4
174.01	187.82	184.21	98.1
348.01	361.82	348.66	96.4
695.88	712,69	680.47	95.5
1,392.12	1,405.93	1,328.45	94.5
2,436.12	2,449.93	2,216.52	90.5
3,480.12	3,493.93	3,163.68	90.6
			X = 94.7 ± 3.1

[a] Furosine content in sample: 13.81 mg/100 g of protein.

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