A Simple and Rapid High-Performance Liquid Chromatographic Procedure for Determination of Furosine, Lysine-Reducing Sugar Derivative

Under mild heat treatment, the interaction of the protein and reducing sugars occurs with ϵ-deoxyfructosyllysine as the product of reactions between free or protein-bound lysine and reducing sugars. Furosine, the acid hydrolysis product of ϵ-deoxyfructosyllysine, was demonstrated to be a good indicator for expressing the extent of the Maillard reaction. A simple isocratic HPLC technique was developed to quantitatively determine furosine. A reversed-phase Lichrosorb RP8 column, a 0.5% NaOAc–HOAc buffer (pH 4.3) mobile phase, and UV detection at 280 nm were utilized. The method was applied on storage samples of dry pet food, powdered meal replacer, formed meal bar, diet bar, dry milk, cheese sauce, and dry gravy. Good correlation between the storage conditions and furosine levels was obtained for all the samples tested.

Nonenzymatic browning is one of the most common deteriorative reactions in food. For quality control purposes, nonenzymatic browning is still mainly determined by organoleptic tests, that is, either by the off-flavor and/or changes in appearance. Unfortunately, the organoleptic tests are not quantitative nor objective. They are time consuming but not very reproducible. Besides, they are sometimes limited to certain types of food products. Some analytical methods, such as a color extraction (Choi et al., 1949) and ion-exchange chromatography (Finot et al., 1981; Hashiba, 1976) utilized in the past either lacked specificity or were time consuming. Recently, HPLC methods have been reported (Moll and Gross, 1981; Moll et al.; 1982) for analyzing some Amadori-type compounds. Due to the low UV absorptivities of these compounds, the off-column peaks could only be monitored by a refractive index detector, thereby restricting the sensitivity of the method. Furosine was first found by Erbersdobler and Zucker (1966) in an acid hydrolysate of overheated milk. Finot et al. (1968) later obtained it by acid hydrolysis of ϵ-deoxyfructosyllysine. The authors used an ion-exchange amino acid analyzer to determine the furosine level in the acid hydrolysates of different types of milk products. The extent of the Maillard reaction has been successfully determined by measuring the number of lysine units present as ϵ-deoxyfructosyllysine with the furosine method. Here a rapid and simple HPLC method is presented to determine furosine. The method is reproducible: deviations between duplicate runs and day to day preparations are within ±4.0% range. Sensitivity is as low as 0.1 μmol of furosine/g of sample. The newly developed method was used to analyze a wide range of storage samples of various dairy products, dry pet food, snack bar, and dry gravy. Furosine levels were shown to vary significantly depending on the storage conditions.

EXPERIMENTAL SECTION

Reagents. Furosine was prepared according to Finot et al. (1968). The prepared furosine was identified and confirmed by the ion-exchange amino acid analyzer and the HPLC method according to its retention time. The mobile phase consisted of 0.5% acetate buffer prepared by dissolving sodium acetate in Millipore-filtered water and adjusting the pH of the solution to 4.3 with glacial acetic acid. HCl (6 N) was purchased from G. Frederick Smith Chemical Co.

Apparatus and Operating Conditions. The spectraphysics Model 3500B liquid chromatograph was used. The chromatograph was equipped with a Hitachi Model 100-30 spectrophotometer as a UV detection unit and was linked to SP 4000 microprocessor for data output. The column used was a Lichrosorb RP8 (4.6 mm × 250 mm, 10 μm) column. The sample was introduced through a 50 μL loop manual Valco injector. The mobile-phase system was eluted isocratically through the column at a flow rate of 2 mL/min. The UV detector was set at a wavelength of 280 nm. The speed Vac concentrator was a Savant Model SVG 100E, and the model was equipped with a one-step heater to 45 °C.

Table I. Reproducibility of the HPLC Measurement

date of preparation	furosine level, mg/g	mean ± SD
1	13.95	
	14.15	14.05 ± 0.10
2	12.94	
	13.07	13.01 ± 0.07
3	13.64	
	13.42	13.53 ± 0.11

Sample Preparation. A total of 0.1–0.5 g of sample containing approximately 20 mg of protein was weighed into a 25-mL screw-cap vial. A total of 10 mL of 6 N HCl was pipetted and the contents were vortex mixed. The vial was capped and heated in an air oven at 110 °C for 20 h. After the vial was cooled to room temperature, the contents were filtered through a glass wool filter into a 10-mL volumetric flask, and the filtrate was made to volume with deionized water. A 2.0-mL aliquot of the hydrolysate was pipetted into a 12 × 75 mm disposable culture tube. The tube was placed in a Savant Speed Vac concentrator, and the solvent was removed by vacuum at 45 °C. The residue was dissolved in 2.0 mL of Millipore-filtered water and was ready for HPLC analysis.

RESULTS AND DISCUSSION

The chromatograms of a control and storage sample (5 months at 45 °C) of powdered meal replacer are shown in Figure 1. To determine optimal HPLC conditions, a series of mobile-phase systems with pH values ranging from 4.0 to 7.0 was tested. It was found that at the low pH value, the furosine peak was cochromatographed with interference peaks and at the high pH value, the furosine peak trailed seriously. The acetate buffer (0.5% at pH 4.3) was determined to give the best separation efficiency. Under these conditions, a typical HPLC analysis is completed in 10 min.

The reproducibility of the HPLC methodology was investigated on a severe heat treated (2 h at 110 °C) powdered meal replacer sample. The data are presented in Table I. As can be see, the method is very reproducible. Deviations between duplicates and day to day preparations are within the ±4% range.

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Table II. Furosine Level in Acid Hydrolysates of Storage Samples

samples	storage conditions	furosine level, mg/g
nonfat dry milk	control	4.83
		4.81
	10 weeks at 45.0 °C	11.58
		11.28
semimoist cheese sauce	4 weeks at 4 °C	0.07
	4 weeks at 45.0 °C	0.14
		0.15
instant dry cheese sauce	8 weeks at 29.4 °C	1.20
		1.15
	8 weeks at 37.8 °C	2.49
		2.54
	8 weeks at 45.0 °C	4.42
		4.69
powdered meal replacer (chocolate flavor)	control	1.84
		1.84
	1 month at 45.0 °C	3.02
		3.06
	3 months at 45.0 °C	4.47
		3.81
	4 months at 45.0 °C	4.84
		4.74
	5 months at 45.0 °C	4.36
		4.32
dry gravy	90 weeks at 22.2 °C	0.42
		0.40
diet bar	control	2.11
		2.15
	55 weeks at 22.2 °C	13.87
formed meal bar	control	0.61
		0.54
	14 weeks at 37.8 °C	5.50
		5.77
dry dog food	control	0.91
	12 weeks at 22.2 °C	1.53
		1.51
	12 weeks at 37.8 °C	3.17
		3.21

Since the Maillard reaction is inherent to food chemistry, its implications should be considered whenever food storage and food processing are studied. Amadori-type compounds (1-amino-1-deoxy-2-ketose) are formed during the first step of non-enzymatic Maillard browning reaction (Nursten, 1981), and ϵ-deoxyfructosyllysine is a representative of this class of compounds. The Amadori compounds are known to be precursors of the color, aroma, and flavor compounds of processed foods. The furosine method can be applied to any food product as long as lysine and the reducing sugars have reacted. In this study, the method was applied to storage samples of a variety of dairy products, snack bars, dry gravy mixes, and dry pet foods. Table II lists the individual storage conditions and the furosine levels. Almost all the storage samples showed significant increases of furosine after prolonged storage and/or elevated temperatures. In the case of the powdered meal replacer samples the furosine level started to drop after 5 months of storage, indicating that the degradation of Amadori compounds may occur after severe heat or storage conditions. The results of Table II strongly indicate that the furosine method may be an indicator of non-enzymatic browning and can be used for a variety of food products. The use of HPLC to determine furosine is fast, simple, and economical. The tedious column regeneration and postcolumn derivatization procedures of the ion-exchange amino acid analysis method are both eliminated. On the basis of the base-line noise and peak responses of the surrounding peaks, the detection limit of the HPLC method is about 0.1 μmol/g of sample, which is superior to that of the ninhydrin-based amino acid analyzer.

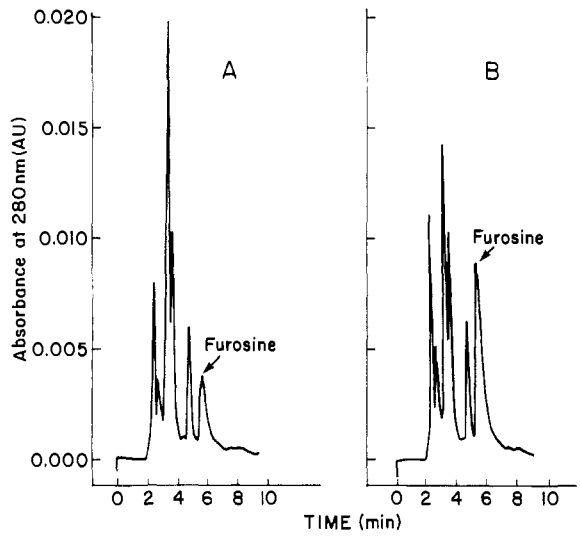

Figure 1. HPLC chromatograms of acid hydrolysates of powdered meal replacer products (chocolate flavor): (A) control; (B) 20 weeks of storage at 45 °C. Chromatographic conditions: Lichrosorb RP8 column; mobile phase, 0.5% acetate buffer, pH 4.3; flow rate, 2 mL/min.

Registry No. Furosine, 19746-33-9.

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Grace H. Chiang

Carnation Research Laboratory
Van Nuys, California 91412

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