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Short Communication

Stability of furosine during ion-exchange chromatography in comparison with reversed-phase high-performance liquid chromatography

J. Hartkopf und H.F. Erbersdobler*

Institut f iir Humanernährung und Lebensmittelkunde der Christian-Albrechts-Universität zu Kiel, Düsternbrooker Weg 17, 2300 Kiel (Germany)

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ABSTRACT

Since a furosine standard became available only recently, arginine was used as the external standard in ion-exchange chromatography (IEC) and 2-acetylfuran in a recently proposed HPLC method. Using the pure furosine standard it was subsequently shown that both procedures produced inexact furosine values. With IEC a substantial decrease in the recovery of furosine of up to 30% was found depending on the elution conditions of pH and temperature that were applied. On the other hand, the use of 2-acetylfuran with HPLC led to an overestimation of furosine content of about 20%. However, correct furosine values were obtained using an IEC elution buffer of pH 4.00 at a temperature of 60°C as was shown by comparison with the HPLC method also based on the furosine standard. These results allow correction of the data obtained in earlier studies and more accurate determination of furosine values in future.

INTRODUCTION

For more than 25 years **ε-N-(2-furoylmethyl)-L-lysine,** named furosine for short, has been used as an indicator of lysine sugar derivatives such as fructoselysine, lactuloselysine and maltuloselysine formed in heated foods in the first steps of the Maillard reaction [1,2]. Furosine analyses are also applied in food science and nutrition, in clinical research and in medical biochemistry (see ref. 2). Furosine is determined by ion-exchange chromatography (IEC) [3,4], HPLC [5–7] and gas chromatography [8,9]. A problem in the determination of furosine was that until recently there was no pure and stable standard available. The furosine peaks were related to peaks of other amino acids such as lysine or arginine.

Recently a reduction in the recovery rate for furosine has been experienced, and therefore the conversion factor used to estimate lysine damage in heated foods was increased [10]. The main change during this time was the use of modem single-column equipment with high-resolving resins running with elution buffer with higher pH values and at higher temperatures. However, the pH value of the buffers known to be important for furosine stability was not increased above pH 6.4. Meanwhile Resmini et al. [7] reported that

* Corresponding author.

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considerable amounts of furosine are destroyed during ion-exchange chromatography, although their newly proposed HPLC procedure using low temperatures and a low pH together with 2-acetylfuran as a helpful substitute for the standard should give better results.

In order to determine the real losses of furosine during IEC analysis a set of experiments were carried out utilizing the recently available pure furosine standard as well as heated lysine-glucose models. Subsequently we compared the results of IEC with those of HPLC, first using, also on HPLC, the pure furosine standard.

EXPERIMENTAL

The furosine standard with a purity >99% was obtained from Neosystem, Strasbourg, France. For initial experiments a sample of furosine was provided by Dr. Finot, Nestec, Vevey, Switzerland. An amino acid calibration standard was obtained from Sigma (Deisenhofen, Germany), lysine hydrochloride from Serva (Heidelberg, Germany), 2-acetylfuran from Aldrich (Steinheim, Germany) and other reagents (all analytical-reagent grade) from Merck (Darmstadt, Germany).

An equimolar mixture of lysine hydrochloride and glucose in an 88% (w/v) aqueous solution was used as a model sample, which was heated for 20 h, and then hydrolysed for 20 h with 7.8 *M* hydrochloric acid at 110°C.

Furosine was analysed using a Liquimat III amino acid analyser (BHS-Labotron, Peissenberg, Germany) as previously described [4], using either a long glass column (170 × 4 mm I.D.) or a short column (50 x 4 mm I.D.) filled with the ion-exchange resin MCI Gel CK10F (Mitsubishi, Japan). The amino acids were detected with ninhydrin with a coil temperature of 115°C. Data acquisition and peak integration were evaluated with an integrator CI-10 from LDC (Gelnhausen, Germany). The elution of furosine was performed in a set of experiments using three different sodium citrate buffer solutions —(1) pH 6.40 (0.96 *M* Na^+), (2) pH 4.70 (1.60 *M* Na^+) and (3) pH 4.00 (1.60 *M* Na^+)— while different column temperatures from 45 to 80°C were applied. Furosine was also analysed with the RP-HPLC method [7] using a C_8 column (Spherisorb 5 C_8 column from Promochem, Wesel, Germany), a detection wavelength of 280 nm and either 2-acetylfuran or furosine as external standard. The HPLC system included a Gradientmaster, constaMetric III/IIIG pumps and a SpectroMonitor D UV detector from LDC (Gelnhausen, Germany), an AS-4000A autosampler from Merck-Hitachi (Darmstadt, Germany), and a C-R4A Chromatopac integrator from Shimadzu (Duisburg, Germany).

RESULTS AND DISCUSSION

To evaluate the influence of conditions during IEC on furosine determination, a furosine standard was analysed using elution buffer with different pH values and different column temperatures. The results of these experiments are shown in Fig. 1. Each value represents an average of six determinations. The peak areas of furosine obtained at pH 6.40 clearly decreased

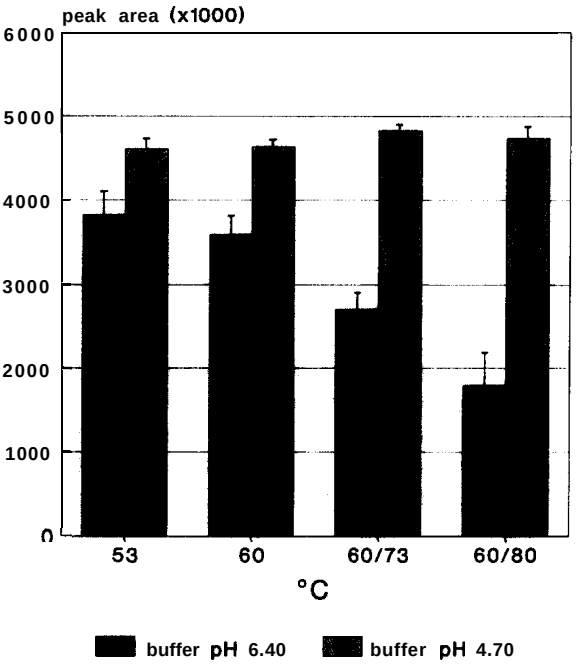

Fig. 1. Comparison of the peak areas of a furosine standard depending on temperature using elution buffers at pH 6.40 and 4.70. 60/73 and 60/80 mean: 30 min at 60°C and 40 min at 73°C or 80°C (pH 6.40) or 20 min at 60°C and 20 min at 73°C or 80°C (pH 4.70).

with increasing column temperature, although the time for elution was shorter at higher temperatures. At 80°C furosine was exposed for 30 min less than at 45°C (elution buffer pH 6.40 in both cases). This indicates destruction of furosine under these conditions. No direct investigation was made for reaction products or fragments of furosine. However, a degradation to lysine was not recognizable, because differences in the lysine peaks could not be detected.

In contrast to the decrease in the furosiue peak areas with an elution buffer pH of 6.40, no significant differences depending on temperature were found at pH 4.70 (Fig. 1). Generally, the peak areas at pH 4.70 were higher than at pH 6.40. Also, the lower standard deviations at pH 4.70 indicated a better stability of furosine at this pH.

Fig. 2 compares the IEC method using a long (170 x 4 mm I.D.) and a short column (50 x 4 mm I.D.) and different pH values and column temperatures with the HPLC method. The comparison did not reveal any major differences, the analysed amounts of furosine being quite similar. However, using the long column at 60°C with an elution buffer pH 4.00 resulted in the highest furosine values. Under these conditions clear and well separated furosine peaks were obtained. The quantification was reproducible and correct, as was confirmed by adding a standard to the sample and comparing the furosine levels. The recovery rate was 99%.

The results obtained by IEC are in contrast to the findings of Resmini et al. [7], who found higher values with their HPLC method. In fact, our previous investigations using the HPLC procedure of Resmini also resulted in significantly higher values than with IEC using buffer at pH 4.00 and a column temperature of 60°C. On the other hand, the method of Resmini uses 2-acetylfuran as standard and not pure furosine. The results of more recent experiments show a marked difference between the peak values of equimolar concentrations of 2-acetylfuran and furosine . The 2-acetylfuran standard yielded only 80% of the furosine standard, resulting in an overestimation of furosine when 2-acetylfuran is used as external standard. Moreover, the 2-acetylfuran standard was found to be unstable during storage. By using the pure furosine standard instead, the same results as with the improved IEC technique were obtained, as is shown in Fig. 2.

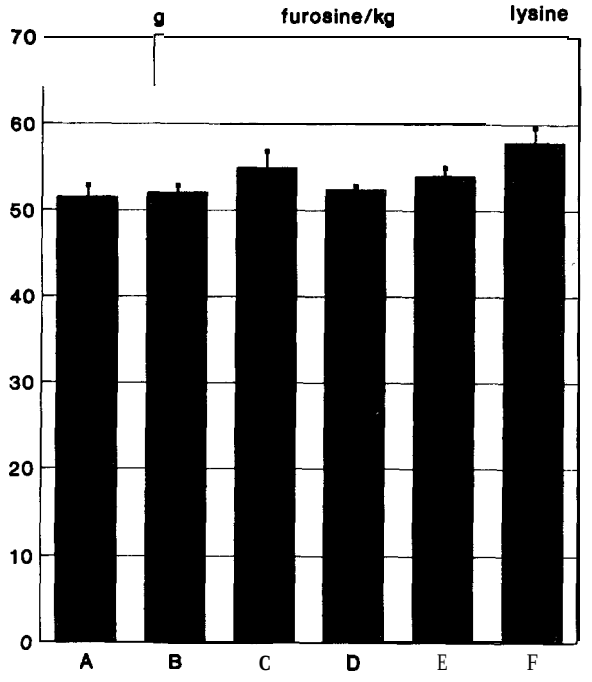

Fig. 2. Comparison of the furosine levels in a heated lysine-glucose model depending on column length, pH of the buffers and temperature. (A) 170 × 4 mm I.D. column, buffer pH 6.40, temperature 45°C. (B) 170x4 mm I.D. column, buffer pH 4.70, temperature 60°C. (C) 170 × 4 mm I.D. column, buffer pH 4.00, temperature 60°C. (D) 50 × 4 mm I.D. column, buffer pH 4.70, temperature 50°C. (E) 50 × 4 mm I.D. column, buffer pH 4.00, temperature 40°C. (F) HPLC method according to Resmini *et al.* [7] but using the pure furosine standard.

Since a pure furosine standard is now available, it was possible to re-examine earlier determinations of furosine, when arginine was used as external standard. Arginine standards analysed in the same way as described above showed no recognizable differences when buffer pH and column temperature were changed. Since the arginine-furosine ratio was not constant, one has to consider that the evaluation of furosine by using arginine as external standard led to an underestimation depending on the analytical conditions applied. But the results of these investigations allow correction of data obtained from earlier studies with higher pH and arginine

as external standard. To determine furosine values more accurately in future we recommend using the modified IEC procedure (elution buffer pH 4.00 column temperature 60°C) or the HPLC method using the now available pure furosine standard.

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REFERENCES

1 H. Erbersdobler and H. Zucker, *Milchwissenschaft*, *21* (1966) 564–568.
2 H.F. Erbersdobler, in M. Fujimaki, M. Namiki and H. Kato (Editors), ***Amino-Carbonyl Reaction in Food and Biological Systems,*** Elsevier, Amsterdam, 1986, pp. 481–491.
3 A. Brandt and H. Erbersdobler, ***Landw. Forsch.***, 28/II (Sonderheft) (1972) 115-119.
4 H.F. Erbersdobler, B. Dehn, A. Nangpal and H. Reuter, ***J. Dairy Res., 54 (1987) 147-151.***
5 E. Schleicher and O.H. Wieland, ***J. Clin. Chem. Clin.*** Biochem., 19 (1981) 81-87.
6 G.H. Chiang, ***J. Agric. Food*** Chem., 31 (1983) 1373-1374.
7 P. Resmini, L. Pellegrino and G. Batelli, ***Ital. J. Food Sci., 3*** (1990) 173-183.
8 W. Büser and H.F. Erbersdobler, ***J. Chromatogr., 346 (1985) 363-368.***
9 J.A. Dunn, J.S. Patrick, S.R. Thorpe and J.W. Baynes, ***Biochemistry, 28 (1989) 9464-9468.***
10 H.F. Erbersdobler and A. Hupe, Z. ***Ernährungswiss.***, 30 (1991) 46-49.