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HPLC determination of tryptophan in foodstuffs using barium hydroxide hydrolysis at 140°C

Short Communication

J. Landry and **S. Delhaye**

Laboratoire de Chimie Biologique, INRA, CBAI, Centre de Grignon, Thiverval-Grignon, France

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Summary. Hydrolysis of samples of food and feedstuffs with 2.7 N $Ba(OH)_2$ at 140°C for 4 h was tested for the recovery of tryptophan. On the basis of 100% yield for the tryptophan content, corresponding to samples determined after 16h-hydrolysis at 125°C, the recovery averaged 98.7 ± 0.9% SD or 99.4 ± 2% depending on how the bulk or aliquot of hydrolysate was analysed (conventional or simplified procedure). Tryptophan can be assayed by high performance liquid chromatography and fluorescence detection within 8h, 4h-hydrolysis at 140°C and a simplified procedure.

Keywords: Amino acids – Tryptophan-assay – Food – Feedstuffs – Barium hydroxide hydrolysis – High temperature – HPLC

Introduction

In a previous study we have developed a procedure for the assay of tryptophan which has been shown to be quantitative. Tryptophan 98–102% from lysozyme added to samples was recovered and identical values for tryptophan were obtained from cereal samples hydrolysed with barium hydroxide or with pronase (Delhaye and Landry, 1986, 1992). The first step of the procedure involved a 10–16 h hydrolysis of samples at 125°C in the presence of 2.7 N barium hydroxide . Oxygen was excluded from the hydrolysis mixtures by transferring them in autoclave when water is boiling and by allow the vapor to escape for 5 min. prior to the rise in temperature. The second step requirred the neutralization of hydrolysate and the clarification of suitable dilution prior chromatographic analysis of tryptophan (third step). It has further been simplified by performing an approximative dilution of a very small volume of liquid

phase of cold hydrolysate containing 5-methyltryptophan, as internal standard, with the pH 4.5 chromatographic mixture and by omitting clarification. The simplified and conventional procedures, when applied to the same hydrolysate, were found to give identical values for tryptophan (Landry and Delhaye, 1992). On the other hand, an investigation about the influence of time and temperature of hydrolysis on the release of tryptophan has shown that a 4h-heating at 140°C in the presence of barium hydroxide enabled the complete recovery of tryptophan from wheat and soybean when it was quantitated through the conventional procedure without the help of internal standard (Delhaye and Landry, 1993). The present study examines the possibility of extending the simplified procedure to tryptophan assay from hydrolysates obtained at 140°C. In addition, α-methyltryptophan was tested as possible internal standard since its use has been proposed recently (Bech-Andersen, 1991).

Material and methods

Samples representing an assortment of foods and feedstuffs whose the nitrogen and tryptophan contents have already been determined (Landry and Delhaye, 1992). The equipment and experimental conditions were as described previously (Landry and Delhaye, 1992). Hydrolysis was carried out by heating sample (50 mg protein) in the presence of 4.2 g of barium hydroxide, 1.5 ml of 0.002 M 5-methyltryptophan and 6.5 ml of water at 125°C for 16 h using a bench-top autoclave (a 10 litre sterilizer from Certoclav, A-4050 Traun, Austria). Oxygen exclusion was obtained as noted in the "introduction" section. A very small volume (5 μl) drawn from liquid phase of hydrolysate cooled at 0°C was diluted with 1.5 ml of a pH 4.5 buffer prior to chromatography (simplified procedure). The bulk of hydrolysate was acidified to pH 3, diluted and clarified prior to chromatography (conventional procedure). HPLC of treated hydrolysate was performed with a system consisting of a Water U6K sample injector, a Waters M510 pump and a reversed phase (Nova-Pak C18, Waters) column (4 μm, 15 × 0.39 cm) maintained at 45°C with a circulating bath. Tryptophan and 5-methyltryptophan were detected from their native fluorescence with a Shimadzu RF 535 fluorescence monitor, working at 285 (excitation) and 345 (emission) nm, and quantitated using a Waters M 730 data module. The modifications with reference to previous works (Landry and Delhaye, 1992) are:

- the addition of 1.5 ml of mixture of α- and 5-methyltryptophan, 0.002 M respectively, to the sample prior to hydrolysis;
- the hydrolysis was performed at 140°C for 4h;
- the sampling of a second aliquot (5 μl) of hydrolysate which was further diluted with 1.5 ml of a mixture of hydrochloric and acetic acid at the respective final concentrations of 0.01 and 0.1 M;
- the eluent consisting of 100 ml methanol and 900 ml of buffer (0.007 M sodium acetate adjusted to pH 4.5 with glacial acetic acid);
- the column temperature was maintained at 50°C.

The singlepoint calibration was performed from a mixture of tryptophan, α- and 5-methyltryptophan to which the same conditions were applied as to the samples.

Results and discussion

Chromatographic separation of tryptophan, α- and 5-methyltryptophan

Fig. 1 depicts a typical chromatogram pertinent to the separation of tryptophan, α- and 5-methyltryptophan which were eluted isocratically at about 2.9, 3.8 and

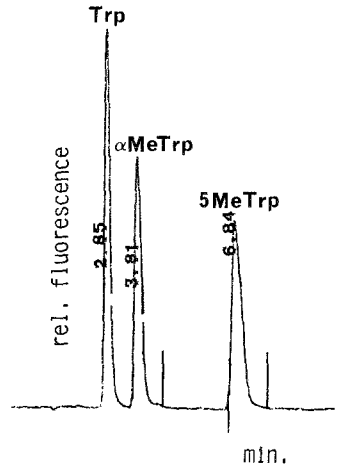

Fig. 1. Chromatogram of tryptophan (*Trp*), α-methyltryptophan (*αMeTrp*) and 5-methyltryptophan (*5MeTrp*) obtained by HPLC and fluorimetric detection. Numbers indicate retention time in minutes

6.8 min. Our chromatographic conditions, compared with those developed by Nielsen and Hurrell (1985) or by Beech-Andersen (1991), led to a faster elution without altering the resolution between tryptophan and α-methyltryptophan. The ratio of the retention times for the amino acids in question is independent of the composition of the mobile phase used.

Determination of tryptophan from barytic hydrolysates obtained at 140°C

Two sets of experiments were performed (Table 1). In the first the samples were hydrolyzed in duplicate and tryptophan was assayed by the conventional procedure involving the adjustment of the alkaline hydrolysate to pH 3 in order to eliminate traces of barium carbonate. In the second, the samples to which α- and 5-methyltryptophan was added, were also hydrolysed in duplicate, and tryptophan was assayed using the simplified procedure. The results reported in Table 1 are expressed on the basis of 100% yield for the tryptophan contents of corresponding samples (16h-hydrolysis at 125°C). The percentages, as evaluated using the conventional procedure, were found to range from 96.8 (maize) to 99.7 (soybean) with a mean of 98.7 and a standard deviation of 0.9, indicating a complete recovery of tryptophan. The latter also applied when using the simplified procedure with 5-methyltryptophan, percentages averaged 98.1 and 99.4 according to whether the final pH of the hydrolysate was 4.5 or 3.0. However, more variable values were to be noted since percentages ran from 94.3 to 102.0 for pH 4.5 dilution and from 96.7 to 102.6 for pH 3.0 dilution. In contrast, results obtained using α-methyltryptophan were found to be unsatisfactory due to a 90% (pH 4.5) and 93% (pH 3.0) recovery of tryptophan. This discrepancy may originate from the presence of a fluorescent compound which coelutes with α-methyltryptophan. The increased temperature used for hydrolysis is believed to be responsible for the formation of the coeluting compound in samples. It is worth noting that in a similar experiment, with 5 samples

Table 1. Comparison of the tryptophan content of foods and feedstuffs as determined after hydrolysis at 125 and 140°C

		Hydrolysis	125°C, 16h		140°C, 4h				
		Procedure	Conventional		Conv.	Simplified			
		pH dilution	3.0		3.0	3.0		4.5	
		Standard[a]	Tryptophan		Trp	5MT	αMT	5MT	αMT
Samples	P/DM %[b]	Tryptophan	% DM	Recovery %[c]	Recovery %				
Maize	10.4		0.085	100	96.8	97.1	90.0	94.3	87.8
Wheat	10.9		0.160	100	98.2	100.3	93.2	99.5	91.0
Barley	12.2		0.195	100	98.7	100.4	94.5	100.1	92.7
Sorghum	12.8		0.151	100	98.6	97.2	92.7	95.9	90.2
Wheat Bran	18.8		0.316	100	98.9	101.5	92.4	101.2	88.6
Pea	24.8		0.258	100	98.7	96.7	92.7	94.3	90.2
Soybean	48.4		0.740	100	99.6	98.2	94.5	97.6	92.1
Whey	12.8		0.322	100	99.7	100.5	89.8	98.1	82.6
Fishmeal	72.8		0.847	100	98.7	102.6	99.4	102.0	96.6
Mean					98.7	99.4	93.2	98.1	90.2
SD					0.9	2.1	2.1	2.8	3.8

[a] Standards are typtophan (Trp), 5-methyltryptophan (5MT) and α-methyltryptophan (αMT)
[b] Crude protein (P = Nitrogen × 6.25) and tryptophan are expressed as dry mass (DM). Data from Landry and Delhaye, 1992
[c] Recovery is expressed on the basis of 100% yield for the tryptophan content of corresponding samples, determined after a 16 h – hydrolysis at 125°C

hydrolysed at 125°C for 16h, the simplified procedure applied to pH 3.0 led to tryptophan recoveries averaging 98.3 ± 1.9% SD and 100.5 ± 1.6 % depending on whether α-or 5-methyltryptophan was taken as internal standard (data not shown). On the other hand, decreasing pH of the hydrolysate dilution from 4.5 to 3.0 appeared to promote a slight increase in the recovery of tryptophan and a decrease of the variability (as assessed through the standard deviation) irrespective of the internal standard used.

The results show that a simplified procedure prior to chromatographic separation of tryptophan can be applied to food heated at 140°C for 4h in the presence of 2.7 N barium hydroxide without altering the accuracy in the quantification of tryptophan. The procedure, similar to that developed for hydrolysates obtained at 125°C for 10 or 16h, involved the use of 5-methyltryptophan and the dilution of the hydrolysate with a mixture of hydrochloric and acetic acid. For the first time operating conditions were reported enabling the quantification of tryptophan in complex samples within a day's.

It is interesting to compare the hydrolytic conditions and the data reported here with those published by Lucas and Sotelo (1980) who have used lithium hydroxide. These authors recommend a higher concentration in alkali (4 N), a slightly higher temperature (145°C), a ratio alkali sample (1 ml/25 mg) and a period of heating of 4 or 8h depending on whether the sample was high (64–91%) or low (9–35%) in protein. Under these conditions a 5% destruction

of tryptophan, as evaluated after ion-exchange chromatography and ninhydrin colorimetry, was found. This has led one to regard lithium hydroxide as the most efficient among alkalies compared with barium (2.5 and 4 N) or sodium (4.5 and 10 N) hydroxyde for the liberation of tryptophan. It is believed, that during the 8-h-hydrolysis there is a steady build up of a ninhydrin positive compound which coelutes with tryptophan upon analysis, compensating for losses of tryptophan. Therefore, lithium hydroxide cannot be considered as a better hydrolytic agent for the quantitative recovery of tryptophan in complex materials. This confirms previous findings (Landry et al., 1992; Slump et al., 1991).

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Authors' address: Dr. J. Landry, Laboratoire de Chimie Biologique, INRA-CBAI, Centre de Grignon, F-78850 Thiverval-Grignon, France.

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