Tryptophan Content of Feedstuffs as Determined from Three Procedures Using Chromatography of Barytic Hydrolysates

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Nineteen samples originating from 14 types of feedstuffs were analyzed for tryptophan according to three procedures: The first required barytic hydrolysis at 105 °C under vacuum, chromatography over Sephadex G 10, and ninhydrin colorimetry. The second and the third involved barytic hydrolysis at 125 °C in the absence of air, reversed-phase liquid chromatography, and ultraviolet spectrophotometry or fluorometry. The values obtained with the three procedures for a given sample were identical. Tryptophan from lysozyme added to 11 samples was recovered in a mean yield of 99.7 ± 1.0% as measured by the second procedure.

The loss of tryptophan during protein hydrolysis in the presence of 6 M HCl currently used for amino acid analysis has prompted investigations to specify suitable conditions for the quantitative assay of this amino acid. Among the numerous proposed procedures, one was that developed by Slump and Schreuder (1969) and based on protein hydrolysis with barium hydroxide in the absence of air, followed by acidification of the resulting hydrolysate, chromatographic separation of tryptophan over Sephadex G 25, and its colorimetric assay with ninhydrin. Three variants of this procedure were compared in this study. They yielded identical results for a given sample, and they appear reliable means for assaying tryptophan in feedstuffs as the recovery of tryptophan from lysozyme added to samples averaged 99.7 ± 1.0%.

EXPERIMENTAL SECTION

Samples represented an assortment of 14 feedstuffs. Lysozyme from Merck was 90% pure according to its nitrogen content and spectrophotometric determination. Nitrogen content was determined by a semimicro Kjeldahl method. Tryptophan content was measured through the three following procedures.

Procedure I was performed at Nantes. Meals (600 mg) were placed with 4 g of barium hydroxide octahydrate and 10 mL of 5% thiodiglycol in test tubes (15 × 120 mm) fitted with a male joint and a stopcock. The tubes were immersed in a boiling water bath for 15 min to ensure a good dispersion of baryta. After cooling, they were evacuated with a tap water pump, filled with nitrogen, and evacuated again. This procedure was repeated twice prior to hydrolysis, which was carried out for 16 h at 105 °C. The tube contents, once they were cooled, were transferred into a beaker by repeated washes with 0.5% thiodiglycol, neutralized to pH 3.5 with 4 M HCl using a pH meter, and diluted to 50 mL into volumetric flask. Hydrolysate (1 mL) was applied to a bed of Sephadex G 10 (9 x 350 mm) equilibrated with pH 3.25 citrate buffer (Delhaye and Landry, 1986a). Elution was performed with the same buffer at a flow rate of 45 mL/h. After 30-min elution, ninhydrin reagent was mixed at a flow rate of 22.5 mL/h with the column effluent. Absorbance was read at 570 nm (Slump and Schreuder, 1969; Delhaye and Landry, 1986a). One analysis required 2 h (1 h for elution and 1 h for regeneration). The time can be reduced by half by using two columns.

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Procedure II was carried out at Grignon. It has been described recently (Delhaye and Landry, 1986b). It involved sample (300 mg) hydrolysis in the presence of barium hydroxide octahydrate (4.2 g) at 125 °C for 16 h, air being excluded. Tryptophan from acidified hydrolysate was isolated by reversed-phase high-performance liquid chromatography on Nova Pak C_{18} (Waters Associates) column (3.9 × 150 mm) and quantified by spectrophotometry at 280 nm. Suitable regeneration of column after every tryptophan separation has been described to avoid possible interferences of ultraviolet-absorbing impurities with the spectrophotometric assay. When recovery experiments were performed, 2 mL of solution containing 5 mg/mL of lysozyme was added to samples prior to hydrolysis. Three analyses/h can be performed.

Procedure III, carried out at Grignon on another hydrolysate, differred from procedure II only by the quantitation of tryptophan from its native fluorescence through a Water Associates Model 420 fluorescence detector and by the absence of column regeneration after every chromatographic run. The emission and excitation wavelengths were 280 and 340 nm, respectively. The specificity of detection was assessed from 10 successive chromatographic runs involving a 10-μL injection of sorghum hydrolysate every 11 min followed by an isocratic elution with a mixture of 50 mL of methanol and 950 mL of buffer, which was 0.07 M sodium acetate containing 0.25 mL/L of triethylamine and adjusted to pH 4.5 with glacial acetic acid, at a flow rate of 0.5 mL/min. The coefficient of variation was found to be 1.5%. A linearity in detector response (peak area) was seen in the range of 20-2000 μg of tryptophan injected. Five samples can be analyzed/h by taking into account a 5-min washing of column with 50% methanol and a 5-min equilibration with eluting medium after 10 chromatographic runs to avoid column alteration.

RESULTS AND DISCUSSION

Table I presents the nitrogen and tryptophan content of 19 samples, expressed on constant dry weight basis, together with the tryptophan recovery from lysozyme added to 11 samples and assayed by procedure II. Comparing procedures I and II showed identical values for 8 out of 18 samples and insignificant (according to a Student's test) differences for 10 other ones. Similarly, there were no differences in the values obtained by procedures II and III, and consequently in those found with procedures I and II.

Tryptophan from lysozyme was recovered in a mean yield of 99.7 ± 1.0%, indicating that neither losses nor incomplete liberation occurred in the presence of barium at 125 °C. This percentage is very close to recovery values (100.4 ± 5.4) reported by Scheuermann and Eckstein

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Table I. Tryptophan in Feedstuffs

	% crude protein (N × 6.25)[a]	g Trp/100 g dry matter			Trp,[d] %	g Trp/100 g crude protein (av)
		I[b]	II[c]	III		
barley	13.1	0.174	0.174	0.174	100.9	1.13
maize	9.4	0.075	0.075	0.076	99.1[e]	0.80
sorghum	11.0	0.136	0.136	0.135	nd[f]	1.24
wheat	13.1	nd	0.149	0.148	nd	1.14
wheat bran	17.0	0.280	0.280	0.275	98.9[e]	1.64
horse bean	27.8	0.353	0.353	0.348	nd	1.26
lucerne	19.2	0.275	0.286	0.280	100.8	1.47
lupine	38.2	0.409	0.397	0.405	98.8	1.05
pea 1	26.7	0.326	0.326	0.326	99.7	1.22
pea 2	26.8	0.284	0.284	0.288	nd	1.06
soybean meal 1	51.5	0.850	0.839	0.850	99.9[e]	1.64
soybean meal 2	54.9	0.845	0.856	0.851	nd	1.55
soybean meal 3	51.7	0.884	0.874	0.894	nd	1.71
soybean meal 4	49.9	0.719	0.709	0.714	nd	1.43
rapeseed meal	39.7	0.540	0.552	0.544	99.6	1.38
peanut meal	54.3	0.494	0.494	0.494	100.0[e]	0.91
fish meal 1	77.1	0.632	0.655	0.640	99.8[e]	0.84
fish meal 2	67.5	0.675	0.648	0.662	nd	0.98
meat meal	63.8	0.402	0.415	0.396	99.2	0.64

[a] On dry matter basis. [b] Procedure. [c] Values given are the average of duplicate analysis on one hydrolysate. [d] Calculated from the known amount of lysozyme added to samples. [e] From Delhaye and Landry (1986b). [f] Undetermined.

(1986) for glycine–tryptophan and leucine–tryptophan–methionine peptides hydrolyzed alone in the presence of barium hydroxide under nitrogen at 120 °C whereas recovery of tryptophan added to maize was 93.8 ± 4.6% only. From this and the overall agreement between the three procedures for the tryptophan content of any sample, it can be concluded that the hydrolysis conditions, as used in the procedure I, did not affect the yield.

On the above background the notable discrepancies in the tryptophan content seen among the four samples from soybean and the two samples from pea and fish meal cannot be attributed to experimental conditions but to the quality of proteins present in these samples, as shown the data of Table I expressed on a constant-protein basis. In this context, the comparison of the present data with those reported from one sample of a given material can be approximate only. So, from a collaborative study, by the method of Miller (1967) in which tryptophan of sample hydrolyzed with barium hydroxide is assayed colorimetrically by reaction with *p*-(dimethylamino)benzaldehyde, Williams et al. (1982) reported for barley, containing 9.6% protein, tryptophan contents (g/100 g of protein) of 1.13 (uncorrected and identical with the value given in Table I) and 1.27 (corrected). The discrepancy between the latter value and that reported in the present study may be accounted for by differences in protein content since this parameter and tryptophan content in protein are inversely related in cereal grain (Delhaye and Landry, 1986a). On the other hand, tryptophan content of soybean meal containing 50.4% protein was reported to be 1.29% (uncorrected) and 1.52% (corrected and close to the value given in Table I) whereas that of fish meal with 73.6% protein was estimated to 1.07% (uncorrected and close to one of both values given in Table I) and to 1.27% (corrected). Regarding maize, examination of literature data in a previous paper (Delhaye and Landry, 1986a) has revealed a wide variability in the determined tryptophan contents of grains originating from normal varieties and having similar nitrogen contents, suggesting that colorimetric assays are not entirely satisfactory.

It is also of interest to compare the data recently reported by Werner (1986) since material hydrolyzed in the presence of lactose and 4 M lithium hydroxide under nitrogen at 110 °C for 24 h was analyzed under conditions close to those of procedure III. The hydrolysis of samples for different lengths of time showed the absence of tryptophan destruction. This is confirmed by the tryptophan content of wheat with 13.6% protein, which was identical with the value given in table. Soybean and fish meals with 43.4 and 62.7% protein were found to contain 1.32 and 1.17% tryptophan, respectively.

In conclusion, the near identity between the values of tryptophan content of any sample obtained by three procedures and the high recovery of tryptophan from lysozyme added to samples show that hydrolysis with barium hydroxide in the absence of air is likely one of most suitable means for releasing tryptophan from protein and avoiding its destruction, thereby assessing its content in feedstuffs through chromatography and ultraviolet spectrophotometry or fluorometry.

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