Quantitative Determination of Tryptophan in Food and Feedstuffs: Practical Considerations on Autoclaving Samples for Hydrolysis

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Hydrolysis of wheat flour and soybean meal in the presence of 2.7 N barium hydroxide at 110 °C for 16 h, at 125 °C for 8 h, or at 140 °C for 4 h led to a complete release of tryptophan, as determined by a conventional procedure involving hydrolysate neutralization by HCl, reversed-phase high-performance chromatography, and quantitation by fluorometry. These data together with the features of two types of benchtop autoclaves used for hydrolysis allow one to specify the more practical conditions for this process, constituting an essential step for the quantitative assay of tryptophan.

INTRODUCTION

A step of our analytical procedure for quantitation of tryptophan involves the hydrolysis of sample in the presence of barium hydroxide and without oxygen (Delhaye and Landry, 1986; Landry et al., 1988). Autoclaving is an efficient way to achieve these conditions. Interfering oxygen is thoroughly removed from hydrolysis medium and sample by purging autoclave at 100 °C for a short time after the sample transfer to the boiling autoclave and before the rise in pressure (Landry and Delhaye, 1992; Landry et al., 1992). Alkaline hydrolysis by autoclaving, with or without purging at 100 °C, has been used by many workers (Miller; 1967; Slump and Schreuder, 1969; Buttery and Soar, 1975; Jones et al., 1981; Slump et al., 1991; Bech-Andersen, 1991). None, however, has specified equipment features, which, within the time constraints of the working day, govern the experimental conditions of hydrolysis. The present paper thus briefly describes two commercial benchtop laboratory autoclaves and reports the kinetics of tryptophan release from two food samples heated with 2.7 N barium hydroxide at three temperatures. These studies better define practical conditions of hydrolysis.

EXPERIMENTAL PROCEDURES

Material. Two types of benchtop autoclaves are commercially available in France. Type I is represented by a 10-L autoclave, sold as a steam sterilizer under the trade name Certoclav. It is manufactured by Kelomat (A-4050 Traun, Austria). This apparatus works at two preadjusted temperatures, 125 and 140 °C. It has an unconfined gasket guaranteeing tightness between body and cover. The possibility for this gasket to move laterally in both directions and to leak at temperature below 100 °C enables one to automatically stop heating of the apparatus without causing a vacuum inside it.

The type II autoclave is portrayed by a 8-L autoclave sold as a steam sterilizer or pressure cooker under the trade name L'Auto-Thermos. This apparatus works at two temperatures, 110 and 125 °C. It has a confined gasket (O-ring) to ensure tightness between body and cover. Decreasing the temperature to below 100 °C creates a vacuum in the apparatus, promoting vigorous boiling of hydrolysis medium with pronounced sample losses.

Autoclave heating is performed on a hot plate.

Method. Wheat flour with a nitrogen content of 1.71% on a dry basis and soybean meal with N = 7.37% were used as food samples low and rich in protein, respectively. Tryptophan was evaluated by a conventional procedure involving sample autoclaving in the presence of 2.7 N barium hydroxide and in the absence of internal standard, hydrolysate neutralization with 6 N HCl, tryptophan isolation by reversed-phase high-performance chromatography, and quantitation by fluorometry (Delhaye and Landry, 1986; Landry et al., 1992).

Table I. Recovered Percentages of Tryptophan from Wheat Flour and Soybean Meal Hydrolyzed at Three Temperatures for Different Times[a,b]

hydrolysis time, h	110 °C		125 °C		140 °C	
	wheat	soybean	wheat	soybean	wheat	soybean
2	76.8	75.7	81.6	87.1	98.8	96.2
4	88.6	90.7	89.6	93.9	98.8	99.6
8	97.4	96.3	99.3	98.9	99.8	101.1
16	99.2	99.4	100.0	100.0	99.6	100.0
20	100.2	99.4				
24	99.7	100.0	99.8	100.8		

[a] Reference (100) corresponds to the tryptophan recovered after 16 h of hydrolysis at 125 °C. [b] Mean of values from duplicate hydrolyses.

RESULTS AND DISCUSSION

Tryptophan in wheat flour and soybean meal, as evaluated by the conventional procedure from eight hydrolyses (per food sample) performed at 125 °C for 16 h on different days, was found to average 1.08 and 1.17 g/16 g of nitrogen, respectively. Percentage recoveries ranged from 98.8 to 101.2% for wheat and from 97.3 to 102.7% for soybean; these values were not significantly different from the initial tryptophan level at a 95% confidence level. Table I reports the recovery percentages for samples hydrolyzed at 110, 125, and 140 °C for various lengths of time.

The data in Table I show complete liberation of tryptophan was achieved within 16 h at 110 °C, within 8 h at 125 °C, and within 4 h at 140 °C. With similar conditions of hydrolysis, Slump and Schreuder (1969) reported that 8 h at 120 °C thoroughly liberated tryptophan from maize and soybean; yields from 4- and 6-h hydrolyses were not significantly different from that determined for 8 h. Nevertheless, in recent years Slump et al. (1991) increased the hydrolysis temperature to 130 °C without changing the duration.

It is noteworthy that tryptophan liberation from barytic hydrolysis is parallelled by amino acid liberation from 6 N HCl hydrolysis. Acid hydrolysis at 145 °C for 4 h yields comparable results with that at 110 °C for 24 h (Gehrke et al., 1985).

Our results (Table I) also show that the time required to reach the maximum recovery of tryptophan is the same for wheat as for soybean. It therefore is independent of

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protein concentration of sample. In contrast, Lucas and Sotelo (1980), working with 4 N LiOH at 145 °C, found maximum tryptophan yield after a hydrolysis of 4 h for protein-rich samples (64–91%) and 8 h for low-protein material (9–35%), such as wheat flour. This difference would originate from lysinoalanine or other ninhydrin-positive compounds (amino sugars) cochromatographed with tryptophan on ion exchanger under the conditions used by the authors. Such an interference has been noted by Hugli and Moore (1972). It could also explain the complete barytic hydrolysis of wheat flour in 4 h at 110 °C, as reported by Knox et al. (1970).

Finally, our data (Table I) show that there is no degradation of tryptophan when length of hydrolysis is extended to 24 h at 125 °C or to 16 h at 140 °C (which corresponded with about 32 h at 125 °C). This emphasizes the great stability of this amino acid in the presence of barium hydroxide.

From the foregoing the following conclusions can be drawn:

(1) A 4-h hydrolysis at 140 °C combined with a simplified procedure similar to that described for the treatment of hydrolysate prior to chromatography (Landry and Delhaye, 1992) allows one to analyze tryptophan within 1 working day. However, it is necessary to assess the stability of 5-methyltryptophan, used as an internal standard, at this temperature before such a procedure is applied.

(2) An 8-h hydrolysis at 125 °C is not attractive at first sight since it takes up most of a working day. Nevertheless, doing such a hydrolysis with the type I autoclave is a versatile procedure, since hydrolysis can be started at any time of the working day and stopped at any time of the night. The autoclave can then cool for the rest of night (or weekend, considering the great stability of tryptophan in barium hydroxide). We use such a procedure routinely in our laboratory. To ensure complete release of tryptophan from any sample, a hydrolysis time of 10 h is selected.

(3) Overnight (16–18 h) hydrolysis at 110 °C can be performed in a less expensive and less versatile type II autoclave.

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