Food Chemistry **49** (1994) 95–97
Analytical Methods Section

Determination of tryptophan in feedstuffs: comparison of sodium hydroxide and barium hydroxide as hydrolysis agents

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(Received 4 October 1992; accepted 14 April 1993)

The tryptophan content of nine samples of food and feedstuffs was determined using two procedures differing mainly by the hydrolysing agent. For a given sample hydrolyses were conducted at the same time and in the same autoclave at 125°C for 16 h after a purging of the apparatus at 100°C for 5 min. $Ba(OH)_2$ hydrolysis yielded the highest values for tryptophan. As for NaOH hydrolysates, the tryptophan recovery evaluated from data obtained with $Ba(OH)_2$ hydrolysates and taken as reference varied from 74·5 to 98·2% with a mean of 88·8%. These values are identical to those reported in literature for recovery of tryptophan added to samples prior to their autoclaving in $Ba(OH)_2$ on NaOH but without purging at 100°C. The tryptophan recovery corrected from losses of added 5-methyltryptophan varied from 95·3 to 106·8% with a mean of 102·3%. The efficiency of autoclave purging at 100°C for the quantitative recovery of tryptophan from $Ba(OH)_2$ hydrolyses would be due to a thorough deoxygenation of medium through boiling.

INTRODUCTION

In a recent study three methods, developed by Slump & Schreuder (1969) (1), by Hugli & Moore (2) and by Holz (1972) (3), for the determination of tryptophan have been compared for evaluating the content of this amino acid in pure proteins and plant material (Delhaye & Landry, 1992). These methods involve protein hydrolysis in the presence of barium hydroxide (1) or sodium hydroxide (2) or pronase (3), isolation of released tryptophan by gel filtration (1) or by ion exchange chromatography (2) or not (3), and its colorimetric assay with ninhydrin (1, 2) or *p*-dimethylaminocinnamaldehyde (3). The results, in agreement with most literature data, have shown that the tryptophan recovery was higher with method (1) although the same hydrolysis conditions, namely 16 h autoclaving at 125°C after a deoxygenation of medium through a 5 min autoclave purging at 100°C, were used with both alkalis. However, the hydrolyses were performed separately and the released tryptophan was determined in different ways. The present investigation was undertaken to increase confidence in the previous results by hydrolysing the same samples simultaneously in the presence of barium hydroxide or sodium hydroxide and by assaying the released tryptophan under the same conditions. In addition, 5-methyltryptophan (5-Metrp) was used as an internal marker.

Food Chemistry 0308-8146/93/$06.00

MATERIAL AND METHODS

Samples represented an assortment of nine foodstuffs.

Tryptophan determination from 1·35 M $Ba(OH)_2$ hydrolysis was performed according to the simplified procedure described by Landry & Delhaye (1992). The only change concerned the hydrolysate dilution: 10 μl of supernatant was mixed with 1 ml of acid solution (0·01 M HCl, 0·1 M acetic acid) in order to destroy any traces of barium carbonate.

Tryptophan determination from 4·2 M NaOH hydrolysis was carried out as follows: a sample containing 50 mg of protein was weighed in a 30 ml disposable polypropylene tube. Ten molar NaOH (2·5 ml) then $0{\cdot}86 \times 10^{-3}$ M 5-methyl-DL-tryptophan (3·5 ml) were added to each sample tube. Capped tubes were vortexed then put into a boiling bench autoclave with tubes containing the same samples but mixed with $Ba(OH)_2$. The autoclave was purged at 100°C for 5 min then heated at 125°C (1·4 bar) for 16 h. Cooled NaOH hydrolysates were neutralised with 6 M HCl (pH 6–7) transferred quantitatively to volumetric flasks and diluted to 50 ml. An aliquot (1 ml) was centrifuged (12 000 g, 5 min) and supernatant (50 μl) was diluted with eluting buffer without methanol. Separation, detection and

quantitation of tryptophan were as described by Landry & Delhaye (1992).

RESULTS AND DISCUSSION

Table 1 pertains to the recovery data obtained after hydrolysis with sodium hydroxide. For each of the nine samples considered it gives: (1) the tryptophan recovery as evaluated by comparing the tryptophan value found from hydrolysis with sodium hydroxide with that determined from hydrolysis with barium hydroxide; (2) the 5-methyltryptophan recovery corresponding to the quotient of the amount of 5-methyltryptophan found after NaOH hydrolysis to that added prior it; (3) the corrected tryptophan recovery calculated from the 5-methyltryptophan losses and corresponding to the quotient of tryptophan recovery by 5-methyltryptophan recovery.

The tryptophan recovery ranged from 74·5 to 98·2% with a mean of 88·8 ± 8·6%. Therefore, it was lower or, at best, equal to that determined from hydrolysis with $Ba(OH)_2$, the percentages of 98·0 and 98·2 being considered not significantly different from 100. Nielsen & Hurrell (1985), applying the procedure of Buttery & Soar (1975), involving autoclaving (without purging) at 100°C in the presence of 6·25 M NaOH for 24 h, reported recoveries of tryptophan added to six food samples ranging from 79·8 to 100·2% with a mean of 86·7 ± 10·3%. This agreement emphasises that the recovery of tryptophan from hydrolysis with NaOH is largely independent of the conditions used for autoclaving.

The recovery of 5-methyltryptophan paralleled that of tryptophan, ranging from 74·5 to 95·8% and averaging 86·1 ± 7·0%. Nielsen & Hurrell (1985) found recoveries between 78·6 and 92·0% with a mean of 85·9 ± 4·8% for the 5-methyltryptophan added to six food samples and hydrolysed in the presence of 4·2 M NaOH at 100°C for 20 h in evacuated tubes. Therefore, the 5-methyltryptophan recovery also is largely independent of conditions used for hydrolysis.

The corrected recovery of tryptophan varied between 95·3 and 106·8%, averaging 102 ± 3·7%. This percentage is slightly higher than that observed by Nielsen & Hurrell (1985) for goat casein hydrolysed together with a chocolate drink powder, but the differences are not significant. It is noteworthy that for three proteins analysed for tryptophan by Bech-Andersen (1991) a mean corrected recovery of 112·2 ± 4·5% or 98·7 ± 2·2% can be evaluated depending on whether 5- or α-methyltryptophan was taken as an internal standard. These values are about 9% higher than those calculated from the data of Nielsen & Hurrell (1985) indicating that the conditions used by Bech-Andersen (1991) for hydrolysis (autoclaving with 4·2 M NaOH at 110°C for 16 h after a 1 h purging at 100°C) led to extra losses of α- and 5-methyltryptophan. In other words the use of α-methyltryptophan as an internal standard would be suitable only for conditions defined in that study.

Table 1. Recoveries of tryptophan and 5-methyltryptophan from NaOH hydrolysates

Samples	Trp % (SD)[a]	5-MeTrp %[b]	Corrected Trp % (SD)[c]
Wheat	94·8 (3·2)	93·2	105·1 (6·5)
Barley	87·8 (3·2)	84·7	103·7 (3·0)
Maize	89·1 (9·3)	84·9	104·9 (0·6)
Sorghum	98·0 (1·9)	93·2	105·1 (6·5)
Alfalfa	74·5 (5·6)	74·5	100·0 (6·1)
Soyabean	98·2 (3·0)	95·8	102·5 (2·8)
Wheat bran	75·5 (3·7)	79·2	95·3 (6·3)
Fishmeal	90·7 (0·8)	91·9	98·7 (2·1)
Meat and bone meal	90·2 (3·8)	84·5	106·8 (1·6)
Mean	88·8 (8·6)	86·6	102·5 (3·7)

[a] Per cent tryptophan recovery for a given sample calculated from tryptophan values determined from NaOH hydrolysate and the mean of values obtained from triplicate $Ba(OH)_2$ hydrolysates. It corresponds to the mean obtained from triplicate NaOH hydrolysates.
[b] Per cent 5-methyltryptophan recovery calculated from the known amount added to samples, and given for information.
[c] Corrected per cent tryptophan recovery calculated from recoveries of tryptophan and 5-methyltryptophan. SD, standard deviation.

It is also interesting to compare the present data with those obtained in a collaborative study in which hydrolysis was conducted with 4 M LiOH, maltodextrin and 5-methyltryptophan in closed tubes or with 1·35 M $Ba(OH)_2$ but without 5-methyltryptophan after a 5 min purging of the autoclave at 100°C (Landry *et al.*, 1992). The corrected tryptophan recovery, as calculated according to the method mentioned above, ranged from 96·1 to 102·4% with a mean of 100·6 ± 1·9% for nine samples. If the lower value is discarded, a mean percentage of 101·1 ± 0·9% is found, closer to those given in Table 1 and by Nielsen & Hurrell (1985). Furthermore, the ratio of 5-methyltryptophan to free tryptophan recovered after LiOH hydrolysis was 103%, equal to the mean of corrected tryptophan recovery reported in Table 1.

The present data together with literature data confirm that hydrolysis with barium hydroxide yields higher values for tryptophan than hydrolysis with sodium hydroxide, in agreement with the complete recovery of tryptophan from lysozyme added prior to hydrolysis as observed by Delhaye & Landry (1986) and by Landry *et al.* (1988). This is due to the thorough deoxygenation of the hydrolysis medium through an effective removal of gas at a temperature close to boiling when the autoclave was purged at 100°C for 5 min (Delhaye & Landry, 1986) or at boiling point when hydrolysis was performed in a closed tube (Landry *et al.*, 1988). If such a treatment is omitted or incomplete (purging at low temperatures), $Ba(OH)_2$ hydrolysis did not lead to quantitative recovery of tryptophan. So, Slump & Schreuder (1969) stated recoveries of free tryptophan of 70–95% or 95–100% depending on the presence or absence of dissolved air in the $Ba(OH)_2$ solution. In the latter case air removal by boiling (as occurs when the autoclave is purged at

100°C) led to recoveries of free tryptophan added to samples of various feeds from 94 to 100% and from 87 to 94% (Slump *et al.*, 1991). Huet & Pernollet (1986) claimed a 94% yield for tryptophan of lysozyme added to wheat flour and autoclaved with unboiled $Ba(OH)_2$ solution after purging (probably at a temperature lower than 100°C). Finally, Miller (1967) reported recoveries varying from 78 to 95% with a mean of 87 ± 5·6% for tryptophan added to nine samples. These values, confirmed by Williams *et al.* (1982) and by Nielsen & Hurrell (1985), are virtually identical to those mentioned for NaOH hydrolysis.

The results show that the procedure of autoclave purging, when applied to NaOH hydrolysis, did not provide a complete recovery of tryptophan. The extent of tryptophan degradation was variable and did not appear to be related to the conditions used for the oxygen removal from the hydrolysis medium. The same was true of 5-methyltryptophan whose losses paralleled those of tryptophan. The correction of data to compensate for tryptophan losses through the recovery of added 5-methyltryptophan, as proposed by Nielsen & Hurrell (1985), resulted in a mean overestimation of 2·3% under the conditions used in the present study.

The preceding considerations for NaOH hydrolysis apply to LiOH hydrolysis.

The differences between alkalis as to the extent of tryptophan recovery could be due to differences in the extent of oxygen removal from the hydrolysis medium, which in turn could be related to the sample solvation. The oxygen present in the hydrolysis medium prior to its removal comes from ambient air and gasses dissolved in liquid or adsorbed on solids (samples, $Ba(OH)_2$). In the presence of 4 M NaOH or 4 M LiOH, ambient and dissolved oxygen can be drawn off from the hydrolysis medium by any treatment involving flushing with inert gas or evacuation or the addition of a protective agent against oxygen or heating at 100°C or a combination of these processes. But this is insufficient to eliminate adsorbed oxygen trapped during solvation of sample constituents other than tryptophan (amino acids and non-protein material). In the presence of 1·35 M $Ba(OH)_2$ the solvation of sample, as assessed by its swelling, is lower and oxygen trapped more loosely could be removed by heating the medium at 100°C. Furthermore, at 100°C deoxygenation would start due to near boiling of $Ba(OH)_2$ solution (boiling point 102°C) in contact with solid particles of sample. By contrast, the same cannot be held for sodium hydroxide solutions since 4 M NaOH boils at 110°C. On this basis a procedure leading to quantitative tryptophan recovery from NaOH or LiOH hydrolysis could be developed. This will be the subject of further investigation.

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