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The stability of tryptophan, 5-methyl-tryptophan and α-methyl-tryptophan during NaOH hydrolysis of selected foods ☆

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ABSTRACT

This study evaluated the use of 5-methyl-tryptophan, α-methyl-tryptophan or synthetic tryptophan to correct for the losses of protein-bound tryptophan in foods during NaOH hydrolysis. Synthetic tryptophan and each protein source was incubated in 4.5 M NaOH containing 5-methyl-tryptophan and α-methyl-tryptophan in nitrogen gas-sparged Teflon vials for 0–144 h at 110 °C. The hydrolysis and loss rates of protein-bound tryptophan, 5-methyl-tryptophan, α-methyl-tryptophan and synthetic tryptophan were predicted using least-squares nonlinear regression. Using 5-methyl-tryptophan or synthetic tryptophan to correct for hydrolytic losses of tryptophan overestimated the tryptophan content by 8.2–19% and −0.3–8.8% respectively, while correction using α-methyl-tryptophan underestimated tryptophan by between 0.2% and 8.1% across the protein sources. Correction using α-methyl-tryptophan or synthetic tryptophan was more accurate than using 5-methyl-tryptophan, but when highly accurate tryptophan composition data are required, least-squares nonlinear regression is the best approach as it removes the need for a hydrolysis correction factor.

1. Introduction

The FAO has recently recommended that the dietary protein quality of foods consumed by humans be determined using the digestible indispensable amino acid score (DIAAS) method (FAO, 2013). Fundamental to DIAAS is the accurate analysis of the indispensable amino acids (including tryptophan) in the foods being examined. Moreover, the amino acid analysis of animal feedstuffs is also important for diet formulation to provide the optimal amino acid nutrition for intensive livestock such as pigs and poultry. The amino acid content of foods and feedstuffs is typically determined using acid hydrolysis to liberate the amino acids from the protein after which the liberated amino acids are separated and quantified. The acid hydrolysis conditions used to hydrolyse proteins are severe (typically 6 M HCl at 110 °C for 24 h in an oxygen-free environment) and not all amino acids can be quantitatively recovered after acid hydrolysis (Fountoulakis & Lahm, 1998; Ozols, 1990; Rutherfurd & Sarwar-Gilani, 2009). Tryptophan is arguably the most acid labile amino acid but some reports suggest that tryptophan is quantitatively stable under alkali conditions (Fountoulakis & Lahm, 1998; Ozols, 1990). Consequently, alkali hydrolysis using either sodium hydroxide (AOAC, 1995; Delgado-Andrade, Rufián-Henares, Jiménez-Pérez, & Morales, 2006; Hanko & Rohrer, 2002; Lucas & Sotelo, 1980; Rutherfurd, Bains, & Moughan, 2012; Yust et al., 2004), barium hydroxide (Landry & Delhaye, 1992; Lucas & Sotelo, 1980) or lithium hydroxide (Lucas & Sotelo, 1980; Rowan, Moughan, & Wilson, 1989; Rutherfurd, Moughan, Lowry, & Prosser, 2008; Sotelo, López-García, & Basurto-Peña, 2007) hydrolysis is most commonly used for determining protein-bound tryptophan.

There can, however, be significant losses of tryptophan during alkali hydrolysis, particularly for foods (Landry & Delhaye, 1996). Accounting for the hydrolytic losses of tryptophan in foods is not straightforward and the losses are often extrapolated from the recovery of synthetic tryptophan (analysed separately from the food sample), or from the recovery of analogues of tryptophan, such as 5-methyl-tryptophan (Delgado-Andrade et al., 2006; Landry & Delhaye, 1992; Rutherfurd et al., 2012; Simat & Steinhart, 1998) and α-methyl-tryptophan (Bech-Andersen, 1991; Landry & Delhaye, 1993; Ravindran & Bryden, 2005), added to the food sample as an internal standard prior to hydrolysis. However, assuming that the recovery of free tryptophan, 5-methyl-tryptophan or α-methyl-tryptophan is the same as for protein-bound tryptophan may be flawed, since the behaviour of free tryptophan and its analogue may be different from that of

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protein-bound tryptophan particularly in complex, and often quite variable, sample matrices such as those present in foods. Quantifying the hydrolytic losses of protein-bound tryptophan is also not straightforward, since to do so requires knowing the actual tryptophan content of the food prior to hydrolysis, which usually cannot be determined without alkali hydrolysis during which hydrolytic losses will occur. To overcome this analytical paradox, some workers (Ravindran & Bryden, 2005) have spiked food samples with purified proteins for which the tryptophan content is known based on amino acid sequence information. However, the latter strategy has limitations since the hydrolytic losses of tryptophan may differ across proteins. In the presently reported study, this impasse is overcome by using a least-squares nonlinear regression (Darragh, Garrick, Moughan, & Hendriks, 1996; Robel & Crane, 1972) to predict the loss rates of protein-bound tryptophan, synthetic tryptophan, 5-methyl-tryptophan and α-methyl-tryptophan directly. The least-squares nonlinear regression method models amino acid hydrolysis and degradation during hydrolysis and permits an accurate prediction of the amino acid content of a protein source (Darragh et al., 1996).

The aim of the study therefore was to investigate the stability of tryptophan (synthetic and protein bound), 5-methyl-tryptophan and α-methyl-tryptophan during alkali hydrolysis using least-squares nonlinear regression to evaluate the efficacy of using the recovery of an internal standard as a predictor for the hydrolytic losses of tryptophan when determining tryptophan in foods and feedstuffs.

2. Materials and methods

2.1. Food protein sources

Eleven protein sources were used and were chosen to vary in their protein, fat and carbohydrate content. Beef muscle, cooked peas, cooked kidney beans, cooked rolled oats, cooked rice, wheat bran were obtained and prepared as described by Rutherfurd, Fanning, Miller, and Moughan (2015). Acid casein and skim milk powder were obtained from Fonterra Co-operative Group, New Zealand. Wheat meal, corn meal and soya bean meal were obtained from Denver stockfoods, Auckland, New Zealand. All protein sources were freeze dried and ground through a 1 mm mesh prior to analysis. Synthetic tryptophan, 5-methyl-tryptophan, α-methyl-tryptophan, maltodextrin were obtained from Sigma–Aldrich (St Louis, MI).

2.2. Analysis

Dry matter, ash, crude protein and total fat were determined in duplicate according to the methods described by AOAC (1995). Total carbohydrate was determined as the difference between the total sample weight and the sum of the moisture, crude protein, ash and the ether extract.

Approximately 50 mg of each protein source was weighed into 14 sets of duplicate 30 ml screw-cap Teflon vials along with 5-methyl-tryptophan (546 μg) and α-methyl-tryptophan (546 μg). In addition a tube containing synthetic tryptophan (306 μg), 5-methyl-tryptophan and α-methyl-tryptophan was also prepared. Eight ml of a freshly nitrogen-sparged solution containing 4.5 M sodium hydroxide and 5% (w/v) maltodextrin (an oxygen scavenger (Jones, Hitchcock, & Jones, 1981; Landry, Delhaye, & Jones, 1992)) was added to each vial and the cap tightly screwed on. The vials were then incubated in a forced-air oven at 110 °C for 0, 2, 4, 8, 16, 20, 24, 32, 40, 48, 72, 96, 120 and 144 h. These hydrolysis times were similar to those used by other workers (Darragh & Moughan, 1998; Darragh et al., 1996; Rutherfurd, Schneuwly, & Moughan, 2007; Rutherfurd et al., 2008). After incubation, the hydrolysates were neutralized with 6 M HCl, made up to volume with deionized water and filtered through a 0.22 μm filter. The tryptophan derivatives were then separated on a reversed-phase C18 column and detected using absorbance at 230 nm. The weight of each amino acid was calculated using free amino acid molecular weights.

The determined concentration of tryptophan for each hydrolysis time ($B(t)$) was then plotted against hydrolysis time and the hydrolysis rate (h, the rate at which the amino acids are released from the protein) and loss rate (l, the rate at which the amino acids are destroyed during hydrolysis) and protein bound tryptophan content (A_o) was estimated using least-squares nonlinear regression analysis conducted using NLIN procedure in SAS (SAS, 2009) and using the compartmental model (Eq. (1)) for describing the liberation and destruction of amino acids during hydrolysis (Darragh & Moughan, 1998; Darragh et al., 1996; Rutherfurd et al., 2007; Rutherfurd et al., 2008):

$$B(t) = \frac{A_o h(e^{-lt}) - e^{-ht}}{h - l} + B_o(e^{-lt}) \quad (1)$$

The determined concentration of synthetic tryptophan, 5-methyl-tryptophan and α-methyl-tryptophan was also plotted against hydrolysis time and least-squares nonlinear regression analysis carried out using NLIN in SAS (SAS, 2009) based on the following model:

$$B(t) = B_o(e^{-lt}) \quad (2)$$

3. Results

The proximate composition of the protein sources is given in Table 1. The protein content ranged from 8.41 to 97.3 g/100 g, total fat ranged from 0.61 to 14.3 g/100 g, ash ranged from 0.761 to 8.16 g/100 g and total carbohydrate ranged from 0.15 to 89.7 g/100 g.

The hydrolysis curves showing the determined tryptophan concentration plotted against hydrolysis time for cooked rolled oats and cooked kidney beans are shown as examples in Fig. 1. Comparable curves for the 5-methyl-tryptophan and α-methyl-tryptophan, which were added to the reaction mixtures prior to hydrolysis, are shown for cooked rolled oats and cooked kidney beans as examples in Fig. 2. The goodness-of-fit between

Table 1
Determined proximate analysis[a] (g/100 g dry matter) of the eleven protein sources.

	Crude protein	Total fat	Total carbohydrate[b]	Ash
Beef muscle	70.8 ± 2.0	14.3 ± 0.23	11.3	3.58 ± 0.09
Cooked kidney beans	22.5 ± 0.74	2.23 ± 0.07	72.9	2.34 ± 0.04
Casein	97.3 ± 0.38	0.980 ± 0.04	0.150	1.70 ± 0.04
Corn meal	8.41 ± 0.68	3.66 ± 0.08	86.5	1.49 ± 0.03
Cooked peas	28.3 ± 0.30	3.93 ± 0.14	65.3	2.58 ± 0.03
Cooked rolled oats	16.3 ± 3.3	4.42 ± 0.15	77.1	2.14 ± 0.07
Cooked rice	8.90 ± 1.8	0.610 ± 0.01	89.7	0.761 ± 0.04
Wheat bran	19.3 ± 0.34	3.46 ± 0.11	70.9	6.38 ± 0.04
Skim milk powder	34.2 ± 3.9	1.01 ± 0.01	57.4	7.42 ± 0.01
Wheat meal	11.1 ± 1.2	1.94 ± 0.05	84.6	2.38 ± 0.08
Soya bean meal	48.0 ± 0.84	2.01 ± 0.08	41.9	8.16 ± 0.06

[a] All analyses were conducted in duplicate.
[b] Total carbohydrate was determined as the difference between the total sample weight and the sum of the moisture, ash, crude protein and the ether extract.