Determination of the tryptophan content of proteins by ion exchange chromatography

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Tryptophan, an essential amino acid, plays an important role in brain function and other regulatory mechanisms. Excess tryptophan is reported to exert atherogenic effects (1). L-tryptophan has also been recently implicated with an outbreak of eosinophilia-myalgia syndrome in USA (1). Its nutritional and toxicological importance emphasise the need for reliable analytical methods for the determination of tryptophan in foods. A method for the analysis of the tryptophan content of foods employing alkaline hydrolysis followed by quantitation of tryptophan in the hydrolysates by ion-exchange chromatography has been developed and tested on lysozyme (Sapphire BioScience, Alexandria, NSW) and a range of proteins.

This procedure is based on hydrolysis with sodium hydroxide in teflon containers under an atmosphere of nitrogen. Samples containing 40-50 mg protein were dispersed in 10 mL of 4.2 M sodium hydroxide containing 0.15 mM 5-methyl tryptophan as an internal standard. At this time the internal standard was also added to the standard tryptophan. Soluble starch (20 mg) was added to samples containing little or no starch as a protective agent. A drop of 2-octanol was added to prevent frothing. The medium was then sonicated, flushed with nitrogen, cooled, evacuated and purged with nitrogen. The process was repeated until all the air was removed from the samples. The samples were then hydrolysed at 120°C for 15 h, cooled, acidified to a pH of 6.5 with HCl, diluted to 50 mL with sodium citrate buffer of pH 6.5, centrifuged and filtered through a 0.2 μm nylon 66 filter membrane. Tryptophan and 5-methyl tryptophan was separated by high performance liquid chromatography on a sodium cation exchange column, using a Shimadzu amino acid analysis system. Aliquots of the sample hydrolysates or standard mixtures of tryptophan and 5-methyl trypytophan were injected onto the column and eluted isocratically with sodium citrate buffer of pH 9.3 at a flow rate of 0.5 mL/min and a column temperature of 65°C. O-phthalaldehyde was used for postcolumn derivatization and fluorimetric detection of amino acids. Separation was accomplished in 20 min without any interference from other compounds in the hydrolysate.

The recovery of tryptophan from lysozyme was greater than 95% and this procedure has proved useful for the routine analysis of cereals, grain legumes and a range of animal feedstuffs. The tryptophan contents (g/kg air dry basis) of the foods were determined to be as follows: wheat, 1.37 (range, 1.23-1.62); maize, 0.62 (range, 0.52-0.87); sorghum, 1.11 (0.89-1.33); triticale, 1.04; barley, 1.23 (range, 1.20-1.40); lupin, 2.76 (range, 2.70-2.82); peas, 2.08 and casein, 10.31. These values compare well with those reported in the literature (2).

1. Doyle EM, Steihart CE, Cochrane BA. Food Safety 1993. New York: Marcel Dekker Inc, 1993.
2. Friedman M, Cuq JL. Chemistry, analysis, nutritional value and toxicology of tryptophan in food. J Agric Food Chem 1988;36:1079-93.