Improved amino acid analysis of feedstuffs

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Advances in analytical chemistry mean that dedicated instruments perform amino acid analyses with greater accuracy than was previously possible. Using novel sodium citrate buffers, data on up to 25% reduction in run times, and improved peak resolutions were achieved using the Biochrom 30 Amino Acid Analyser.

Amino acids play an essential role in the nutritional composition of feedstuffs, however for many years diets were formulated to meet the requirements of crude protein rather than specific amino acids.

Improved analytical techniques and cost-effective commercial production of individual amino acids now allows nutritionists to provide a more precise feed formulation. This allows the full amino acid value of a protein source to be maximized leading to better animal nutrition and health, and long-term economic benefits to the industry.

Accuracy is essential in the determination of feed amino acids, however advances in HPLC analysers have emphasized speed of chromatography at the expense of resolution of peaks. Using the Biochrom 30 Amino Acid Analyser, we present data using a novel sodium citrate buffer system for the analysis of amino acids from complex hydrolysates such as food and feedstuff samples.

Experimental conditions:
The analysis was performed on a Biochrom 30 Sodium System using a 20 cm Oxidised Feedstuff High Performance PEEK column and post-column Ninhydrin photometric detection with dual-wavelength measurements. Chromatographic control, detection, and safety systems were performed via a PC with fully integrated software. The ninhydrin flow rate was set at 25 ml/hr and the buffer flow rate at 35 ml/hr. The system operated using a novel buffer chemistry formulated for this application which is composed of 4 sodium citrate buffers of increasing pH and molarity. As no derivatization was necessary the samples were loaded directly on to the Biochrom 30 Amino Acid Analyser instrument

Results:
Figure 1 (below) shows an overall improvement in amino acid peak resolution using the new buffer system. Specifically, better separation is achieved for amino acids such as cystine, valine, isoleucine and leucine. Additional amino acids such as taurine, 2,6-diaminopimelic acid , β-alanine, glucosamine, galactosamine, and hydroxylysine can also be resolved using this system.
The complete separation of 30 amino acids is achieved in less than 50 minutes (64 mins. injection to injection) and a smoother baseline is observed at both wavelengths, avoiding baseline changes incurred by the buffer changes.

Conclusions:
• The ability to separate up to 30 amino acids from a feedstuff sample allows precise feed formulation to be achieved to ensure optimal animal nutrition.

• A decrease in individual run times means that 8 extra runs can be performed each day providing a significant increase in throughput.

• Smoother baseline, particularly under cystine, allows small amounts of cystine to be accurately quantified.

• The same set of 4 sodium citrate buffers can be used for both the analysis of oxidised protein hydrolysates and protein hydrolysates allowing flexibility of the sample type and ease of use of the instrument.

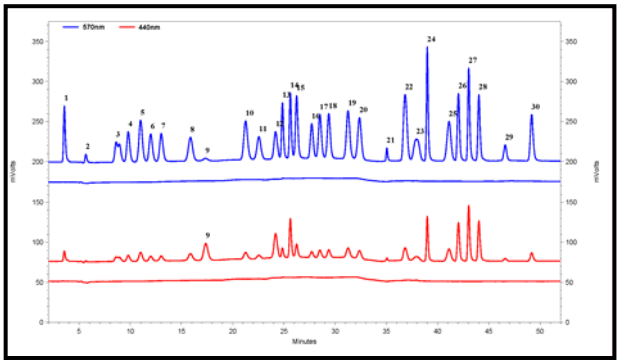

Figure 1: *Chromatogram of a standard mixture (concentration: 5 nmol/20uL for most of the amino acids) and sodium citrate loading buffer, 20 uL loaded. Detection at 570 nm and 440 nm. 1 = cysteic acid, 2 = taurine, 3 = methionine sulfoxide, 4 = aspartic acid, 5 = methionine sulfone, 6 = threonine, 7 = serine, 8 = glutamic acid, 9 = proline, 10 = glycine, 11 = alanine, 12 = cystine, 13 = valine, 14 = 2,6-diaminopimelic acid, 15 = methionine, 16 = isoleucine, 17 = leucine, 18 = norleucine, 19 = tyrosine, 20 = phenylalanine, 21 = β-alanine, 22 = glucosamine, 23 = galactosamine, 24 = histidine, 25 = tryptophan, 26 = hydroxylysine, 27 = ornithine, 28 = lysine, 29 = ammonia, 30 = arginine.*

References
(1) EC Commision Directive 98/64/EC (1999)
(2) The Biochrom Handbook of Amino Acids, edited by Mike Davies