B30-24 Amino Acid Composition Analysis in Alternative Protein Foodstuff

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Introduction

As concern for global food security and the impact of traditional animal agriculture on the environment grows, so too does the market for alternative proteins, be they plant-based, mycoproteins, insect derived or cultivated meat.

There are multiple applications in the preparation of alternative protein products for market where Amino Acid Analysis is recommended, the key areas being taste[1], analysis of the quality and digestibility of the proteins[2], and accurate analysis of total protein content[3].

In this application we analysed and compared the amino acid profiles of a supermarket brand beef burger to a well-known brand plant-based burger.

Experimental Details

Sampling.

The Biochrom instrument can generate accurate results from samples on the milligram scale or less. However, when examining natural materials, it is preferable to use larger quantities to overcome inhomogeneities in the sample matrix. Availability of material is rarely a problem for foodstuffs, and it was not here, so for this examination we removed 1g [~1%] samples from the items.

Analysis Options.

There is sometimes interest in quantifying *free* amino acids in foodstuffs. This can also identify additives and suchlike, e.g., flavour enhancers like Mono Sodium Glutamate and Aspartame. This is performed by extraction with dilute hydrochloric acid. For meat and meat substitutes the

overwhelming source of amino acids is, obviously, to be found incorporated into the protein and so, in this instance, the extraction test is not applicable.

It is possible to hydrolyse protein to its constituents by simple hydrolysis using concentrated acid. Unfortunately, this process destroys some amino acids such as Cysteine/Cystine, Methionine, and Tryptophan. Inclusive results are obtained by oxidising the samples prior to hydrolysis: this converts the sulphur-containing amino acids to Cysteic acid and Methionine Sulphone which are resistant to acid degradation. We followed this method. Tryptophan may be quantified separately by alkaline hydrolysis. There are several variations to this procedure, and we chose a long-established method using Barium Hydroxide[4].

Procedure.

The products tested were obtained through well-known retailers and manufactured in the UK. Aliquots [1g] of the meat and plant-based burgers were transferred to 50mL round-bottomed flasks. To each was added 5mL of oxidation reagent[5]. The flasks were stored at <4°C overnight. The samples were allowed to warm to room temperature and Sodium Metabisuphite [0.84g] was added cautiously to each to destroy the oxidant. The flasks were fitted with water condensers and placed in a heating bath. Hydrolysis reagent [12mL][6] together with a few anti-bumping granules was added to the flasks and the contents were maintained at a gentle reflux for 24 h. After cooling to room temperature, the contents were reduced to a film under vacuum using a rotary evaporator. The residues were taken up in Loading Buffer[7] transferred to 50mL volumetric flasks. Internal standard[8] 1mL was added and the volume was made up to the line with more buffer.

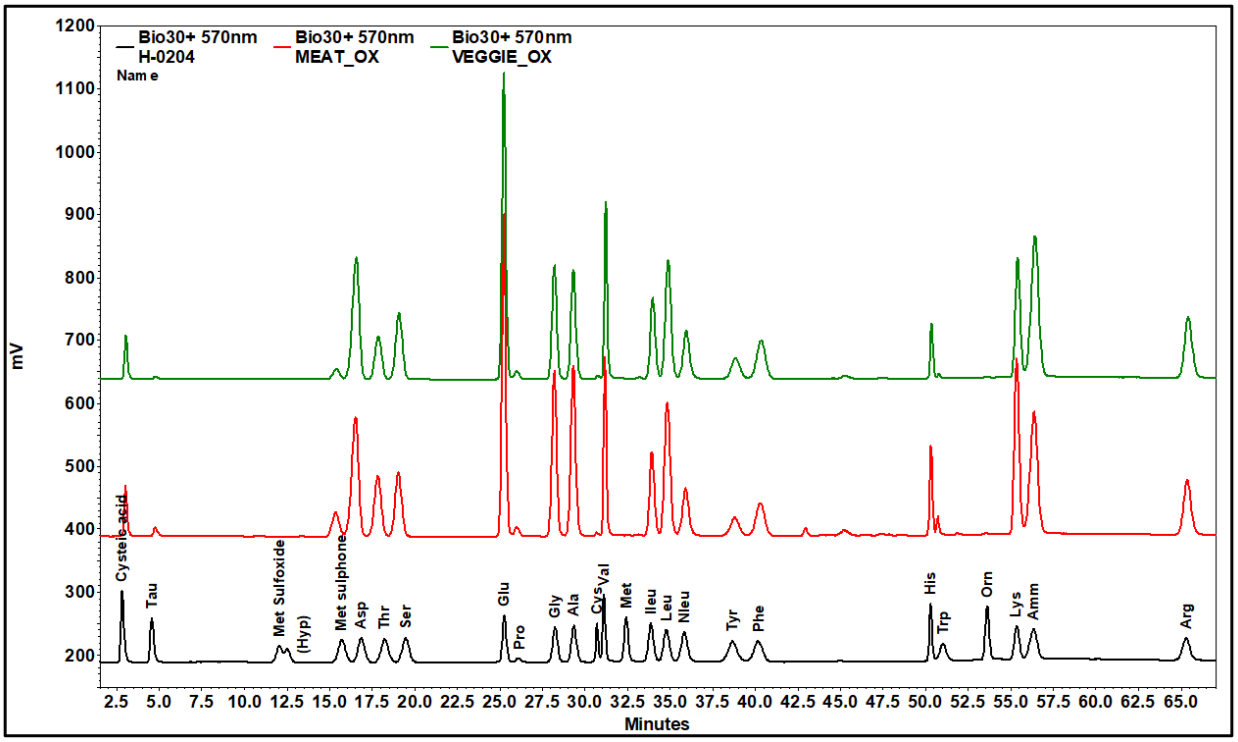
Samples for chromatography [~ 0.5mL] were filtered and transferred into the autosampler in one operation using Uniprep vials[9]. A vial containing buffered Reference Standard with appropriate amino-acids and including an internal standard at 0.5mM was prepared[10]. A vial containing only buffer was also prepared in order to generate a blank run. Chromatography was performed using the 'Sodium Oxidised High Performance' programme on the Biochrom 30+ ion-exchange instrument with appropriate buffers.

For the alkaline hydrolysis 200mg samples were transferred to 50mL round-bottomed flasks along with starch 100mg, barium hydroxide 6g and water 50mL. The samples were heated to reflux overnight and cooled to room temperature. Sufficient hydrochloric acid (conc.) was added to dissolve the barium hydroxide (~3.5mL) and the volume made up to 50mL in a volumetric flask using loading buffer. Samples were filtered and run as previously described.

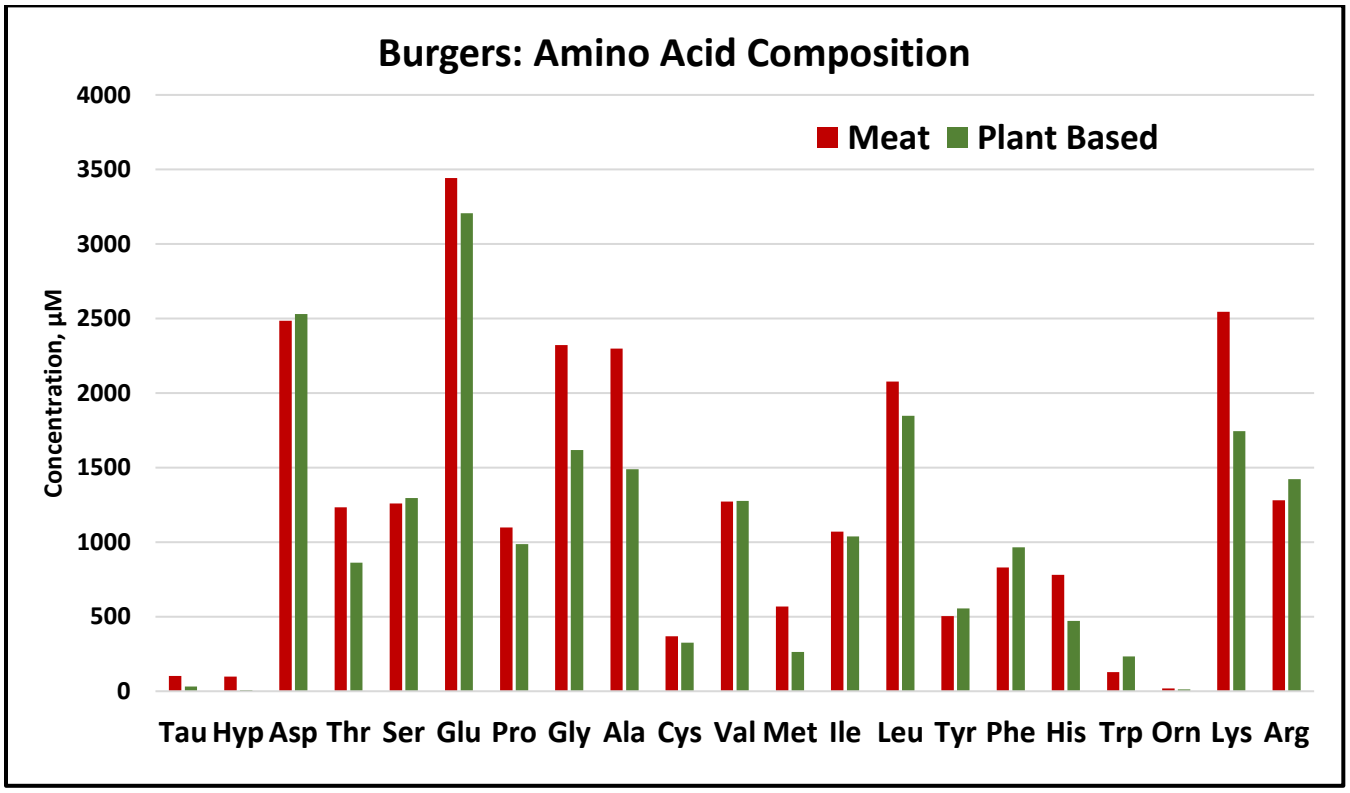
Note. There was no obvious evidence of residual fat in the hydrolysed samples: in cases where this is present in large amounts it is advisable to extract and discard it using a water-immiscible solvent such as light petroleum.

Results

The chromatograms below [570nm channel. Black=standard, red=meat, green= plant-based] show all the amino acids fully resolved within 67 minutes using a standard programme. Cysteine and Methionine were completely converted to their oxidised forms indicating adequate penetration of the oxidation reagent into the sample matrix. Asparagine was converted to Aspartic Acid. Values for Hydroxyproline and Proline were taken from the 440nm channel [not shown].



Peak areas were converted to concentrations using the individual response factors taken from the standard and are presented in the table below.



The two products were quite closely matched in composition, with all the essential amino acids (Thr, Val, Met, Ile, Leu, Phe, His, Trp, Lys) present in both. Concentrations of Threonine, Histidine, Methionine and Lysine however, were significantly higher in the beef product.

By simply multiplying the molar concentration of each amino acid present by the residual molecular weight and summing, it is possible to calculate the protein composition directly. This avoids the errors in using proxy methods such as total nitrogen. Values of 17% and 15% for the meat and plant-based products respectively were obtained, which compared closely with the values stated on the packaging.

Conclusion

Amino Acid Analysis using the Biochrom Bio 30+ is a simple and highly accurate way to separate and quantify constituent amino acids in a variety of traditional and alternative protein-based foods for human consumption.

Results from such analysis can be used in multiple areas of development and manufacturing including monitoring the protein makeup of cell cultures and media, refinement of taste profiles, nutritional analysis for labelling purposes and determination of quality of under recognised rating systems such as PDCAAS.

References

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2. Amare, E, Mouquet-Rivier, C, Servent, A. , Morel, G. , Adish, A. and Haki, G. *Protein Quality of Amaranth Grains Cultivated in Ethiopia as Affected by Popping and Fermentation*. Food and Nutrition Sciences, 6, 38-48. 2015.

3. Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen I-J. *Protein Determination—Method Matters***.** *Foods*. 2018; 7(1):5.

4. A. Dreze and W.S.Reith Biochem. J. 62 (1956) 3P. Also A. Dreze Bull. Soc. Chim. Biol., 42 (1960) 407

5. Prepare a stock solution from Formic acid (100%) 45g, Phenol 250mg and Water 5g.

   Prepare the Oxidation Reagent just before it is required by mixing 18mL of the above stock with 2mL aqueous Hydrogen Peroxide (30%) and stirring at room temperature for 1h. Chill to <4°C before use.

6. Hydrolysis regent is prepared from Hydrochloric acid (conc.) 50mL and Phenol (100mg) made up to 100mL with water.

7. Biochrom Sodium Loading Buffer **#80-2037-57**

8. Internal standard stock solution, 2mM, prepared from Norleucine 131mg in Loading Buffer 50mL.

9. Mini-UniPrep PVDF filters, 0.2µm. Cytiva [Whatman].

10. A working reference standard was prepared from 1-part Biochrom Protein Oxidised Standard 2.5mM, **#80-6002-68**, 1-part Norleucine 2.5mM, and 3 parts Loading buffer.