Amino acid analysis in legumes by Liquid Chromatography - High Resolution Accurate Mass - Mass Spectrometry

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Overview

Purpose: To demonstrate the feasibility of high resolution and accurate mass for profiling amino acids, including quantitation and confirmation in legumes.

Methods: A quick efficient extraction of amino acids in peas was developed alongside a 18 min liquid chromatography - high resolution accurate mass MS (LC-HRAM MS)method.

Results: The high resolution MS provides unequivocal confirmation of amino acids in pea food and feeds, providing great selectivity reducing matrix effects and isobaric interference.

Introduction

Amino acids are both the building blocks of protein and also ubiquitous as free amino acids in a wide range of food and feeds. As the monomeric units of proteins, alpha-amino acids are molecules which contain an amine group (NH2), a carboxylic acid group (COOH), and a side-chain that is specific to each amino acid (R). They have the generic formula H2NCHRCOOH.

Their crucial role in ensuring growth performance in animals and humans means there is an interest in developing efficient, accurate and reliable determination techniques, particularly in the development of food supplements and improving animal feeds.

Analysts at the Food and Environment Research Agency have developed a simple and quick profiling method for 21 free amino acids in legumes using high resolution-mass spectrometry without the need for derivatisation.

FIGURE 1. Harvested legume crops

Methods

Extraction:

1. Take 1g of freeze dried milled sample.
2. Add 10ml of de-ionised water and shake.
3. Centrifuge @ 14,000 rpm for 10mins.
4. Dilute supernatant 9:1 with HPLC water.
5. Run by LC-HRMS.

Instrumentation:

The detection system used was the Thermo Scientific Accela UHPLC system connected to the Thermo Scientific Exactive Orbitrap high resolution mass spectrometer. Source used was ESI in positive mode. Conditions are described in the table below.

TABLE 1. LC-HRMS conditions.

Parameter	Optimised condition
LC Column	ACE AQ (ACT, UK) 150 x 3mm @ 30°C
LC Gradient	0.4 ml/min - 100% water (0.1%HCOOH) to 100% MeCN (0.1%HCOOH) over 15 mins
Injection parameters	5 µl
MS Resolution	Enhanced, 25,000
Polarity	Positive
AGC	Balanced
ESI Sheath gas flow rate	60
Aux gas flow rate	10
Sweep gas flow rate	0
Capillary temperature	350 °C

ThermoFisher Exactfinder and Xcalibur software was used for data processing – both in a quantitative and screening application. Quantification of the acids was carried out using solvent standards and / or a standard addition approach.

Standards, chemicals and reagents

Optima LCMS grade methanol, acetonitrile, and water (Fisher Scientific, UK) were used as mobile phases, for analysis and preparation of standard mixtures and washing of the injection port. To acidify mobile phases HPLC grade formic acid (Fisher Scientific, UK) was used.

Amino acid standards were from Sigma Aldrich, UK.

FIGURE 2. Bench top HRAM Exactive with OrbiTrap Technology

Results

A full scan MS method has been developed for amino acids in legumes using enhanced MS resolution (25,000).

The enhanced resolution combined with high mass accuracy(<5ppm) limits the interference of the matrix.

Using the ExactFinder software data processing can be undertaken using a targeted "Quantitative" approach and / or a separate (non) targeted search for peaks or interest (screening approach). Table 2 describes the acids of interest. Figure 3 shows an example Extracted Ion Chromatograms for tryptophan using Exactfinder in "quan" mode. Figure 4 shows the extracted ion chromatograms for all analytes using a layout in Xcalibur qual browser. Figure 5 displays a screenshot of Exactfinder in "screening mode" finding the amino acid cysteine and correctly predicting its formula. Figure 6 describes the linearity achievable with this method when trying to accurately quantify glutamine in matrix by standard addition. A solvent standard curve is also shown for the same analyte.

TABLE 2. List of compounds of interest, their ionised accurate mass, retention time, and linearity in solvent standards.

	M+H	RT (mins)	R^2
Arginine	175.11894	1.60	0.993
Histidine	156.07674	1.58	0.999
Lysine	147.11279	1.54	0.999
Aspartic acid	134.04477	2.05	0.998
Glutamic acid	148.06042	2.02	0.994
Glutamine	147.07641	1.95	0.995
Threonine	120.06551	1.98	0.990
Serine	106.04986	1.91	0.987
Asparagine	133.06076	1.92	0.992
Cysteine	122.0270	2.07	0.991
Glycine	76.03930	1.87	0.988
Cystine	241.03112	1.88	0.994
Proline	116.0706	2.21	0.999
Leucine	132.10189	4.05	0.994
Isoleucine	132.10189	3.80	0.994
Valine	118.08624	2.40	0.992
Alanine	90.05495	1.91	0.961
Methionine	150.05832	3.03	0.995
Tyrosine	182.08116	4.75	0.995
Phenylalanine	166.08624	8.95	0.999
Tryptophan	205.09714	9.30	0.997

Figure 3. An extracted ion chromatogram for tryptophan in pea extract. Screenshot is from Exactfinder "Quan" mode.

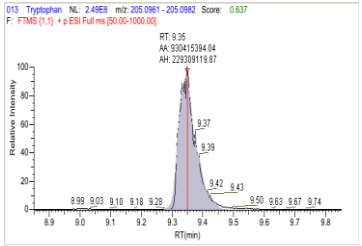


FIGURE 4. Extracted ion chromatograms for all 21 amino acids, shown using Xcalibur software. Mass tolerance set to ± 5 ppm.

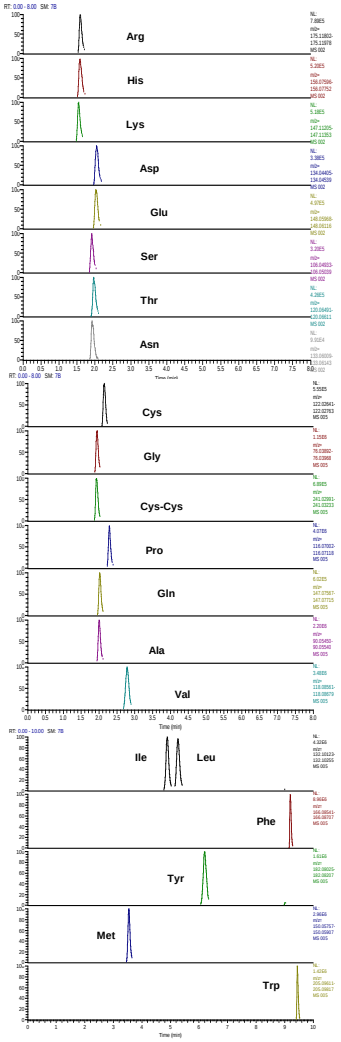


Figure 4. Screenshot of Exactfinder in screening mode. Chromatogram displayed is the amino acid cysteine found in a pea extract, confirmation of the compound is established with a score based on the theoretical isotope pattern.

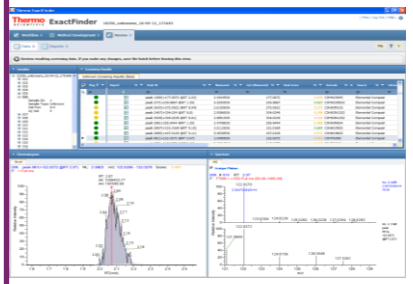
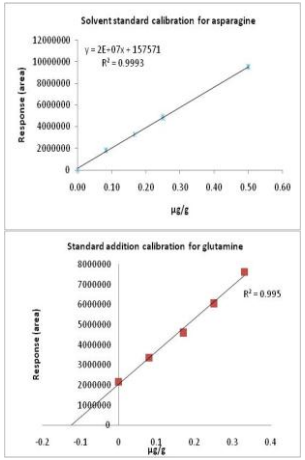


Figure 5. Example calibration curves for asparagine in solvent standards (upper) and glutamine in matrix by standard addition approach (lower).



Conclusion

A method has been developed for the confirmation and subsequent quantification of underivitised free amino acids in pea matrix. The detection is facilitated by High Resolution Accurate Mass LC-MS (HRAM LC-MS)with chromatographic separation by HPLC.

Using Exactfinder all 21 amino acids can be either screened for their presence and / or quantified accurately and efficiently.

The Food and Environment Research Agency