Rapid and Efficient Hydrolysis of Pet Foods for Amino Acid Analysis

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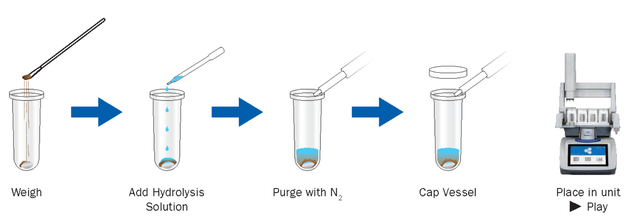
Abstract

The nutritional profile, especially protein content, of pet food is of the utmost importance for its testing and formulation. For the health of our pets, proper nutrition, which includes an optimal blend of amino acids without any deficiencies or excess, is vital. To determine the balance of amino acids in a sample, the protein chains must be first broken down into the constituent amino acids. The classical protocol of amino acid hydrolysis heats the sample to 110 °C in sealed tubes with 6 N HCl to hydrolyze the proteins chains; this method is a manual process that requires lengthy reaction times, often up to 24 hours. In this work, the CEM Discover® 2.0 Microwave Reaction system was used to hydrolyze dry and wet pet food samples using microwave energy in under 30 minutes through higher temperatures and microwave energy. The resulting hydrolyzed samples were derivatized using the Waters AccQ-Tag™ Ultra reagent kit. This derivatization can also be automated with the Waters Andrew+ pipetting robot. Separation and analysis were performed on a Waters ACQUITY UPLC H-Class system with UV detection. This rapid and automated process led to high recoveries of amino acids from pet food samples. This indicates that the CEM Discover 2.0, Waters AccQ-Tag, Andrew Alliance, and Waters ACQUITY UPLC H-Class system with UV detection are suitable for a complete workflow of amino acid analysis of pet foods.

Discover 2.0 Hydrolysis

1. Weigh 50-60 mg of cat or dog food into 35 mL Pyrex vessel with stir bar.
2. Add 5 mL of 6 N HCl with 1% Phenol.
3. Purge vessel with N_2 for 5 minutes.
4. Seal vessel with a Teflon® lined cap.
5. Place prepared vessels in the autosampler.
6. Press Play.



Discover 2.0 Acid Hydrolysis Method Parameters:

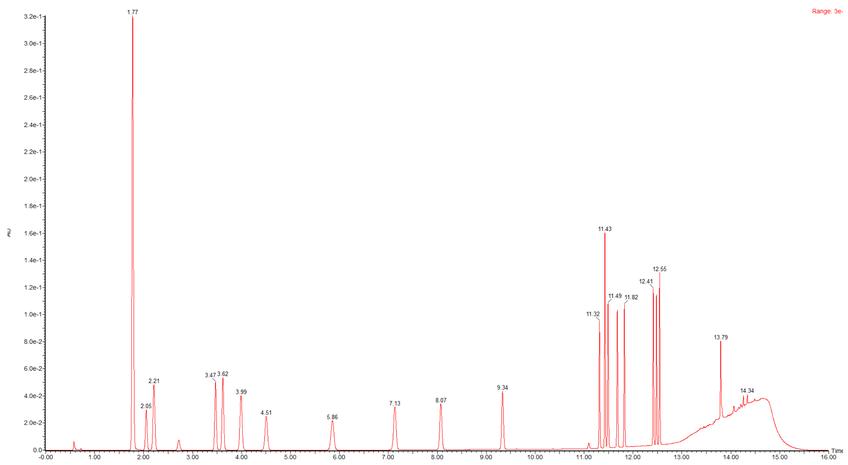
Vessel Type: Pyrex	Pressure: 300 PSI
Control Type: Dynamic	Power: 300 W
Temperature: 160 °C	Stirring: High
Time: 30 min	

Analysis

Post-hydrolysis pre-column derivatization was done using the Waters AccQ-Tag™ Ultra Derivatization kit. This step can also be automated using the Waters Andrew+ robot. The derivatization kit was used following its provided directions as indicated. Then, detection of the derivatized amino acids was done on a Waters ACQUITY™ UPLC™ H-Class with a PDA detector at 260 nm. A Waters AccQ-Tag Ultra C18 column (1.7 µm, 2.1 x 100 mm) set at 55 °C with a 4 µL injection volume was used for analysis. The mobile phases for separation were A: Waters AccQ-Tag Eluent A diluted 10-fold in MilliQ water and B: Waters AccQ-Tag Eluent B.

Time (min)	Flow (mL/min)	%A	%B
Initial	0.4	99.9	0.1
0.54	0.4	99.9	0.1
9.74	0.4	90.9	9.1
11.74	0.4	70.0	30.0
12.04	0.4	40.4	59.6
13.05	0.4	10.0	90.0
13.64	0.4	10.0	90.0
13.73	0.4	99.9	0.1
16.00	0.4	99.9	0.1

Table 1: Gradient used for derivatized amino acid separation



Compound	Time (min)
AMQ	1.77
NH_3	2.05
His	2.21
Ser	3.47
Arg	3.62
Gly	3.99
Asp	4.51
Glu	5.86
Thr	7.13
Ala	8.07

Compound	Time (min)
Pro	9.34
Derivatization Peak	11.10
Cys	11.32
Lys	11.43
Tyr	11.49
Met	11.68
Val	11.82
Ile	12.41
Leu	12.48
Phe	12.55

Chart 1 and Table 2: Chromatogram of derivatized amino acids at 100 pmol/µL with retention times

Results

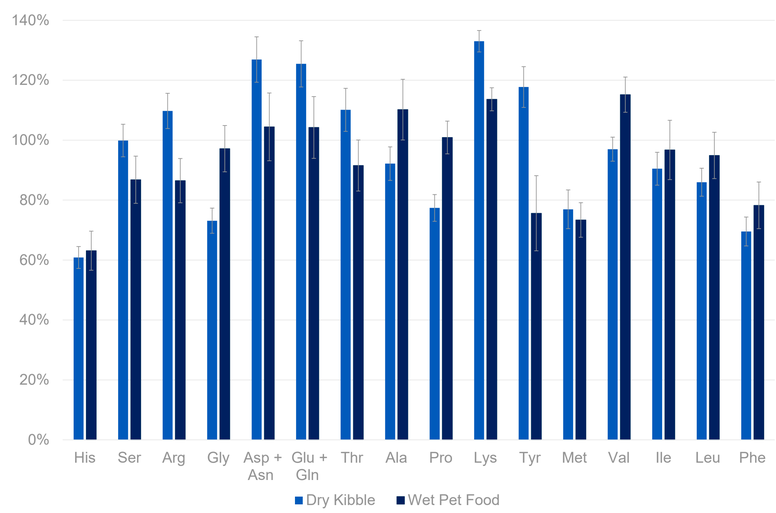
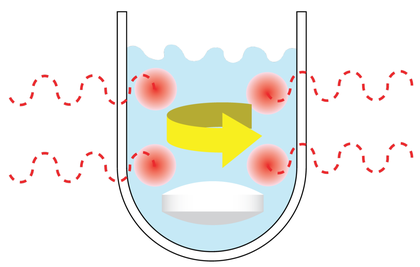


Chart 2: Percent Recoveries for Dry and Wet Pet Food

Key Benefits



- Comparable recoveries to traditional amino acid hydrolysis
- Excellent accuracy and precision
- Reduced reaction times
- Acid and base hydrolysis on one unit
- Cleaner hydrolysates
- Quicker time to analysis

References

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