Using Microwaves for the Rapid and Efficient Amino Acid Hydrolysis of Pet Foods

Introduction

Protein plays a major role in the manufacturing process of wet and dry pet food commodities. Not only does the quality and amount of protein affect the overall cost of production, it also influences consumer interest in the product. One way to assess the quality of a dietary protein is to examine the total amino acid content of the sample by means of amino acid hydrolysis. Classically, proteins are hydrolyzed under acidic or basic conditions for upwards of 24 hours. This treatment breaks the peptide bonds between amino acid residues in proteins, ultimately allowing for the identification and quantification of each amino acid. Long reaction times are required because of slow volumetric heating, erratic heat gradients, and inconsistent reaction yields, characteristic of conventional heating.

Microwave-assisted reactions have been highlighted as an approach to significantly decrease hydrolysis times and increase reaction yields and reproducibility.[1,2] CEM's Discover® 2.0 microwave reactor offers an excellent path for the optimization of protein hydrolysis reactions. Herein, wet and dry pet foods were subjected to traditional acid hydrolysis as well as optimized microwave-assisted hydrolysis conditions. The amino acid content of each sample was determined via pre-column derivatization using the Waters AccQ-Tag™ Ultra Derivatization kit, followed by LC-PDA analysis at 260 nm. Overall, the Discover 2.0 results were comparable to the traditional hydrolysis with key benefits, including shorter reaction times, cleaner hydrolysates, and quicker time to analysis.

Materials and Methods

Reagents and Samples

Chemicals were purchased from commercial suppliers and used without further purification. Solutions used for the traditional and microwave-assisted amino acid hydrolysis were freshly prepared prior to use. All glassware was dried in an air oven at 110 °C for 4 hours. All pet food samples were homogenized and stored in a cool dry place for dry samples or at 4 °C for wet samples until analysis. Between 40 and 50 mg portions of each sample were weighed into their reaction vessels using an analytical balance. All samples were analyzed using the Waters AccQ-Tag Ultra Derivatization kit for LC-PDA analysis at 260 nm.

Traditional Acid Hydrolysis Method

Pet food samples were added to clean and dry 10 mL glass vials, followed by 2 mL of 6 N HCl. Vials were then purged with N_2, quickly sealed, and then placed into a 110 °C air oven for 24 hours. Sample hydrolysates were allowed to cool to room temperature and then passed through a 0.2 µm PTFE filter. The particle-free samples were then neutralized with 6 M NaOH in preparation for AccQ-Tag derivatization.

Discover 2.0 Hydrolysis Method

Hydrolysis Reaction Preparation: A 50 mg portion of each sample was added to a 35 mL Pyrex vessel, equipped with a micro stir bar. A volume of 5 mL of 6 N HCl with 1% phenol (w/v) was pipetted into each vessel. Vessels were purged with a stream of N_2 for 5 minutes, quickly sealed with a Teflon-lined silicon cap, and placed in the autosampler rack for automated placement into the Discover 2.0 cavity. Upon reaction completion, the cooled samples were

neutralized and filtered in preparation for AccQ-Tag™ derivatization.

Method Programming: A one-step Dynamic method was programed in the Discover 2.0 system for the amino acid hydrolysis of pet food samples.

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

Sample Preparation for Analysis

For AccQ-Tag derivatization, 70 µL of borate buffer from the Waters AccQ-Tag Ultra Derivatization kit were added to a complete recovery vial, and then 10 µL of the filtered and neutralized sample were added. The vial was capped and vortexed. Additionally, 20 µL of prepared derivatization reagent from the Waters kit were added to each sample reaction, with the pipette tip directly in the reaction solution. The total reaction was vortexed for 10 seconds and then was heated at 55 °C for 10 minutes, prior to analysis.

Analysis

4 µL of each derivatized reaction were injected onto a ACQUITY™ UPLC™ System hooked to a Waters PDA detector. Separation was done on a Waters AccQ-Tag Ultra C18 column (1.7 µm, 2.1 x 100 mm) using a flow rate of 0.4 mL/min. The column temperature was at 55 °C. The elution gradient is shown in **Table 1**. The mobile phases were A: Waters AccQ-Tag Eluent A diluted 10-fold in MilliQ water and B: Waters AccQ-Tag Eluent B. To determine the amino acid concentrations for each amino acid, Waters Amino Acid Standard (Waters Corporation, Part No. WAT088122) was derivatized at the following concentrations: 1, 5, 10, 25, 50, and 100 pmol/µL, and the resulting linear curve was forced through zero. All analyses were done using the Waters TargetLynx™ software.

Table 1. Gradient Used for Derivatized Amino Acid Separation.

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	30
12.04	0.4	40.4	59.6
13.05	0.4	10	90
13.64	0.4	10	90
13.73	0.4	99.9	0.1
16.00	0.4	99.9	0.1

Results and Discussion

Dry kibble and wet pet food samples were subjected to microwave-assisted amino acid hydrolysis using CEM's Discover 2.0 microwave reaction system. The amino acid content of each hydrolysate was determined via pre-column derivatization followed by LC analysis equipped with a PDA detector set to monitor at 260 nm. Results were compared to those obtained from traditional oven hydrolysis reactions of the same samples. The traditional results were considered to be 100% recovery. **Table 2** depicts the recoveries for each amino acid residue in comparison to their traditional hydrolysis counterpart. All recoveries fall within the acceptable recovery range of 60 to 130% for the dry and wet pet food samples. Furthermore, the microwave-assisted reactions showed greater precision compared to the traditional hydrolysis reactions in terms of standard deviation (as shown in **Figure 1** on page 3).

The Discover 2.0 offers a variety of advantages over traditional amino acid hydrolysis protocols. Added magnetic stirring aids in sample homogeneity, while compressed air cooling brings the reaction vessel to a safe handling temperature within minutes. The Discover 2.0's microwave heating mechanism offers comparable results to conventional heating with increased precision within 30 minutes allowing for decreased time to analysis and greater opportunity for reaction optimization. This approach also represents a safe and automated platform for acid and base amino acid hydrolysis reactions.

Table 2. The Recoveries for Amino Acids in Dry and Wet Pet Foods – Microwave-Assisted vs. Traditional Amino Acid Hydrolysis.

Amino Acid	Dry Kibble	Wet Pet Food
His	60.86%	63.13%
Ser	99.89%	86.80%
Arg	109.74%	86.50%
Gly	73.12%	97.17%
Asp + Asn	126.91%	104.43%
Glu + Gln	125.46%	104.23%
Thr	110.11%	91.54%
Ala	92.17%	110.19%
Pro	77.40%	100.89%
Lys	133.01%	113.64%
Tyr	117.74%	75.61%
Met	76.93%	73.36%
Val	96.98%	115.19%
Ile	90.48%	96.74%
Leu	85.97%	94.90%
Phe	69.53%	78.25%

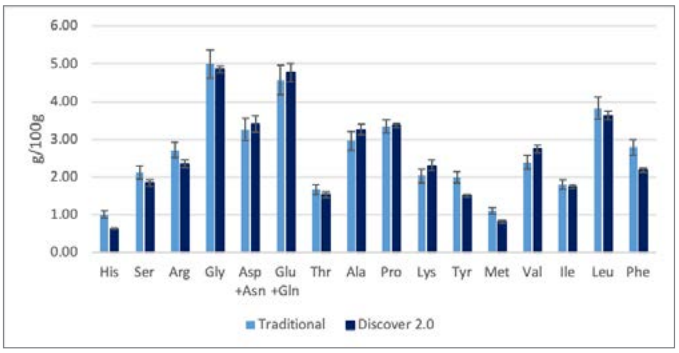

Figure 1. Amino Acid Hydrolysis Results of Wet Pet Food.

Conclusion

This study highlights CEM's Discover 2.0 as an efficient approach for the amino acid hydrolysis of wet and dry pet foods. Overall, the Discover 2.0 results were comparable in terms of accuracy and precision to their traditional oven hydrolysis counterparts, with the key benefits of shorter reaction times, cleaner hydrolysates, and quicker time to analysis.

References

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