The Rapid Preparation of Pet Food for Taurine Content

Introduction

Pets are often thought of as furry (scaled, feathered, etc) members of our immediate family and play a large part in modern life. Pet amenities have ranged from heated beds to specialized grooming, so it is no surprise that their diets have come under greater scrutiny. Dog foods come in a variety of forms and there are many health benefits and risks from different recipes. Recently, grain-free or legume-rich diets have come under fire because of their link to the canine heart disease dilated cardiomyopathy (DM), which can lead to congestive heart failure and ultimately death. DM is caused by a deficiency of taurine, and thus dog's diets must have sufficient taurine.

In this work, the Discover Prep™ system was used to hydrolyze dog food samples to free the amino acids from their bound proteins. Compared to traditional hydrolysis techniques, the Discover Prep uses microwaves to hydrolyze samples, accelerating the timetable of the technique from 24 hours to less than an hour. After hydrolysis, the samples were neutralized and the amino acid content was measured using the Waters AccQ-Tag® kit. The results indicate that, compared to traditional methods, the Discover Prep rapidly hydrolyzed taurine from the proteins in pet food with good accuracy and precision. Samples of grain-free dog food and grain-containing dog food were compared for their taurine content. Discover Prep is an ideal choice for laboratories quantifying taurine and other amino acids in dog food.

Materials and Methods

Reagents and Samples

All reagents were purchased from commercial suppliers. All dog foods were purchased from local pet supply stores. All dog food samples were ground with a commercial kitchen blender. The dog foods were chosen due to their differing primary protein and were marketed as having high taurine content. Three samples were analyzed: Sample 1 was a recipe of salmon, sweet potato, and pumpkin, Sample 2 was a recipe of chicken and sweet potato, and Sample 3 was a recipe of turkey and oatmeal. Hydrolysis solutions were freshly prepared prior to hydrolysis.

The dog food samples were hydrolyzed using the CEM Discover Prep System. The amino acid content of the hydrolysates was determined via pre-column derivatization, followed by UPLC® injection and PDA detection at 260 nm. The Waters AccQ-Tag Ultra Derivatization Kit was used for LC-PDA analysis of the hydrolysates. Results obtained from the Discover Prep sample preparation were compared to the nutritional label as well as the corresponding traditional oven method.

Discover Prep Sample Preparation

A 100 mg sample of dog food was added to a 35 mL Discover Pyrex® vial equipped with a stir bar. The mass was recorded for analysis. For acid hydrolysis, a portion of 5 mL of 6 N HCl containing 1% phenol was then added to each vial. All vials were purged with N_2 for 1 minute and then quickly capped. The samples were run with the following Discover Prep method:

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

Traditional Hydrolysis Sample Preparation

A 40 mg portion of dog food was added to an appropriate glass vial. The mass was recorded for analysis. For acid hydrolysis, a portion of 2 mL of 6 N HCl containing 1% phenol was then added to each vial. All vials were purged with N_2 for 1 minute and then quickly capped. The vials were then placed into an air oven set at 110 °C for 24 hours.

Post Hydrolysis Sample Preparation and Derivatization for Analysis

Acid hydrolysis samples were neutralized with 6 N NaOH and filtered with 0.26 µm PTFE syringe filters. After neutralization, samples were derivatized with the Waters AccQ-Tag kit. A portion of 80 µL of borate buffer from the Waters AccQ-Tag Ultra Derivatization Kit was added to a complete recovery vial. Then, 10 µL of the sample was added, and the sample was capped and vortexed. Then, 10 µL of prepared derivatization reagent from the kit was added to each sample reaction.

Lastly, the reaction was vortexed for 10 seconds and was then heated at 55 °C for 10 minutes prior to analysis.

Analysis

A portion of 1 µL of each derivatized reaction was injected onto a Waters AccQ-Tag Ultra C18 column (1.7 µm, 2.1 x 100 mm), attached to a Waters ACQUITY™ H-Class UPLC® with a Waters PDA detector. A flow rate of 0.4 mL/min was used. The column temperature was at 55 °C, and the absorbance was monitored at 260 nm. The elution gradient used for separation is shown in **Table 1**. The mobile phases were A: Waters AccQ-Tag Eluent A diluted 10-fold in Milli-Q® water and B: Waters AccQ-Tag Eluent B. To create calibration curves for each amino acid, Waters Amino Acid Food and Feed Standard Kit (Waters Corporation, SKU. 186009299) was derivatized at concentrations 1, 5, 10, 25, and 50 pmol/µL. Linear regression was used to analyze the samples.

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
14.74	0.4	90.9	9.1
16.74	0.4	70.0	30.0
17.04	0.4	40.4	59.6
18.05	0.4	10.0	90.0
18.64	0.4	10.0	90.0
18.73	0.4	99.9	0.1
21.00	0.4	99.9	0.1

Results

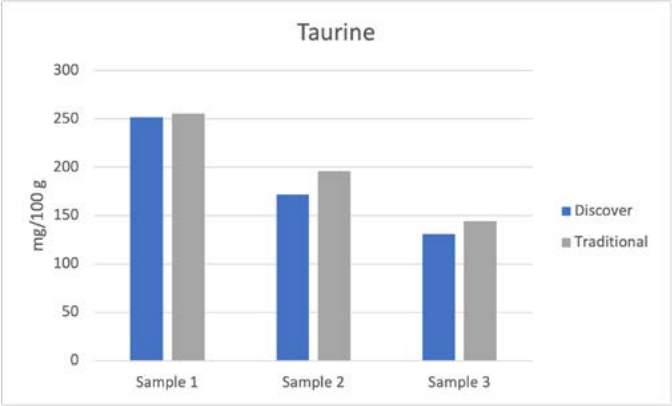

Figure 1. Amount of Taurine Recovered from Discover Prep Hydrolysis

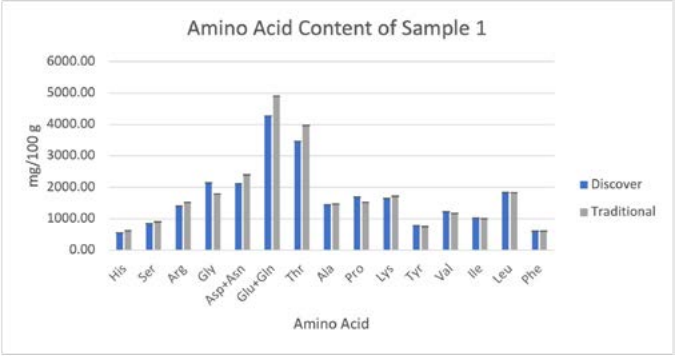

Figure 2. Amount of Amino Acid Recovered from Dog Food

Conclusion

The Discover Prep microwave reaction system was able to successfully hydrolyze the amino acids in pet foods and yield similar results to those produced using a traditional oven hydrolysis. In comparing the amino acid content, both the traditional oven hydrolysis and Discover hydrolysis yielded results with %RSDs less than 10%. In comparing the taurine content of the dog food, Sample 1 had the highest content followed by 2 and 3.

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