The Rapid Preparation of Infant Formula for Amino Acid Analysis

Introduction

Properly creating infant formula with an ideal nutrition profile is absolutely critical. For formula-fed babies, infant formula is often the primary source of nutrition for babies in the first six months of life. The goal of formula is to emulate breast milk as closely as possible. For infants with allergies to breast milk, formula is all the more important, as it can be their sole source of nutrition, and the formula must be free of allergens. The FDA mandates minimum levels for 29 different nutrients in infant formula, including proteins, fats, carbohydrates, and micronutrients like vitamins.[1] Some of these nutrients, such as protein, also have maximum levels. Protein levels in infant formula are also of interest because protein levels are higher in infant formula than in breast milk.[2] Research on how this affects the growth of infants and children is still ongoing, but it highlights the importance of being aware of protein levels in infant formula.

Protein content and amino acid content in food is a hot topic among food manufacturers. Traditionally, the amino acid levels in infant formula are measured using AOAC Official Method 2018.06, which is a typical amino acid hydrolysis oven method requiring 24 hours of treatment. Microwave-assisted methods for amino acid hydrolysis utilize a much shorter time treatment, often less than 1 hour. In this work, the CEM Discover Prep™ microwave-based sample preparation system was used in conjunction with the Waters AccQ-Tag™ Ultra Derivatization Kit to analyze the amino acid content levels in an infant formula standard reference material (SRM). When examining the results, it was determined that the Discover Prep produced results for infant formula hydrolysates that were similar to those determined with traditional methodology, with excellent repeatability. The Discover Prep provides several advantages over traditional methods, such as rapid compressed air cooling and ease of use, and its dielectric microwave heating mechanism provides instantaneous and uniform volumetric heating that helps accelerate the hydrolysis process without compromising recovery or precision.

Materials and Methods

Reagents and Samples

All reagents were purchased from commercial suppliers. A NIST infant formula SRM material was used in this study. The infant formula was reconstituted by combining 1 g of infant formula with 9 mL of water. Hydrolysis solutions were freshly prepared prior to use.

The infant formula samples were hydrolyzed using the CEM Discover Prep. 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 Certificate of Analysis (CofA), as well as the corresponding traditional oven method.

Discover Prep Hydrolysis Method

A 750 µL sample of reconstituted infant formula was added to a 35-mL Pyrex vial, equipped with a stir bar. The mass was recorded for analysis. A portion of 5 mL of 6 N HCl containing 1% phenol was then added to each microwave reaction vial. Vials were purged with N_2 for 1 minute, then quickly sealed with a Teflon® lined silicon cap, and placed in a sample rack, prior to being placed in the Discover Prep cavity. Samples were analyzed in triplicate. A one-step Dynamic method was programmed for the amino acid hydrolysis of the infant formula. The following method parameters were programmed into the Discover Prep:

Acid Hydrolysis
Vial Type: Pyrex
Control Type: Dynamic
Temperature: 160 °C
Time: 20 min
Pressure: 300 PSI
Power: 300 W
Stirring: High

Sample Preparation and Derivatization for Analysis

All hydrolysate solutions were filtered with 0.2 µm PTFE filters and then neutralized with 6 M NaOH prior to derivatization 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 s and then was 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 MilliQ water and B: Waters AccQ-Tag Eluent B. To create calibration curves for each amino acid, Waters Amino Acid Standard (Waters Corporation, Part No. WAT088122) was derivatized at concentrations 1, 5, 10, 25, 50, 100, and 200 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

For the Discover Prep testing, an infant formula SRM was used. When the results were calculated, they were compared to a CofA provided with the material, as well as results obtained from the traditional oven method for amino acid hydrolysis, following AOAC Official Method 2018.06. In **Figure 1** (page 3), the results from the microwave-assisted and traditional preparations, as well as the CofA are displayed. It can be seen that the results for the traditional oven method matched those of the Discover Prep method, with all recoveries falling in the range of 80 to 100%. Furthermore, the standard deviations for the Discover Prep results were smaller in comparison, suggesting improved repeatability. Thus, the Discover Prep data is accurate and precise, when compared to traditional methods.

Conclusion

In this work, the CEM Discover Prep, a microwave-based sample preparation system, was used to hydrolyze infant formula, and it was found to hydrolyze infant formula with high recoveries and excellent precision. The Discover Prep system possesses key benefits over traditional oven methods, such as ease of use, instantaneous and uniform heating, and rapid cooling, while achieving ideal hydrolysis performance.

References

[1] Food and Drug Administration. The Office of the Federal Register (OFR) of the National Archives and Records Administration (NARA), and the U.S. Government Publishing Office (GPO). 21 CFR 107. Infant Formula: The Addition of Minimum and Maximum Levels of Selenium to Infant Formula and Related Labeling Requirements. https://www.federalregister.gov/documents/2013/04/16/2013-08855/infant-formula-the-addition-of-minimum-and-maximum-levels-of-selenium-to-infant-formula-and-related (accessed Nov 16, 2022).

[2] Bo Lönnerdal. Infant Formula and Infant Nutrition: Bioactive Proteins of Human Milk and Implications for Composition of Infant Formulas. *The American Journal of Clinical Nutrition*, Volume 99, Issue 3. [Online] **2014**, 712S–717S. https://academic.oup.com/ajcn/article/99/3/712S/4577485 (Accessed Nov 16, 2022).

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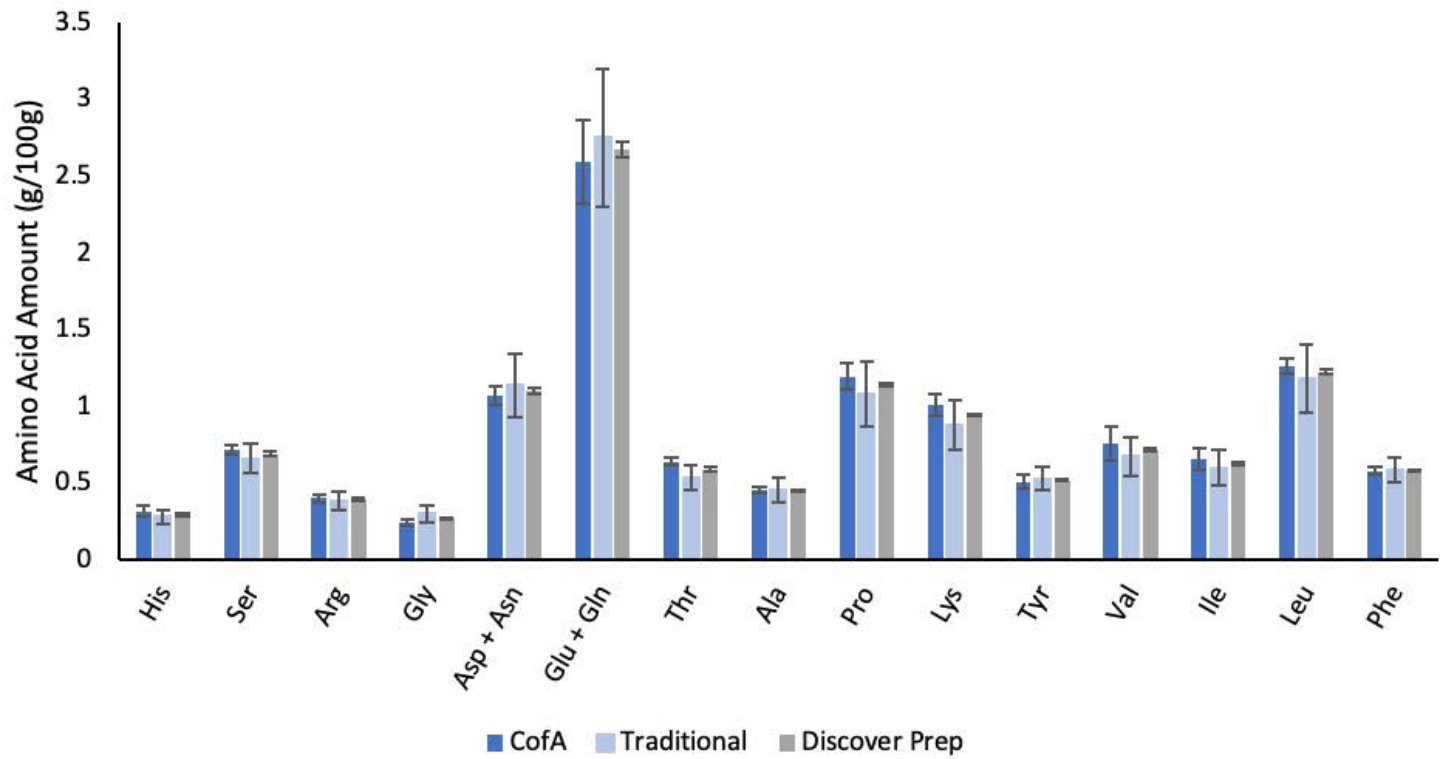
Figure 1. Discover Prep Data Comparison to Traditional Amino Acid Hydrolysis and SRM Certificate of Analysis (CofA)

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