The Rapid Preparation of Vegan Cream Cheese for Amino Acid Analysis

Introduction

With rising concerns surrounding the environmental impact of animal farming, animal welfare, and the nutrition of traditional meat and dairy products, plant-based alternatives are becoming a mainstay to grocery stores, restaurants, and retailers. However, the rapid growth of this industry and the acceptance of these products by consumers has led to a gap in the abilities of regulators and standards groups to monitor and evaluate the efficacy of current analytical techniques. From in-house proximate analysis and nutritional label testing to adhering to FDA requirements on the level of contaminants and more, everything associated with analyzing alternative protein products still needs to be formally defined. One such area includes the identity and quantity of the amino acids that make up the protein in these products.[1]

In order to determine the amino acid content of a sample, proteinogenic amino acid residues must first be released from their protein-bound form. There are several methods available to accomplish this; however, the classical method is to hydrolyze the sample in the presence of 6N HCl at 110 °C for 18-24 hours. This lengthy reaction time represents a significant bottleneck in amino acid analysis. Alternatively, the utilization of microwave heating offers a variety of advantages compared to conventional heating mechanisms. Namely, the dielectric microwave heating mechanism allows for instantaneous and uniform volumetric heating. In contrast, conventional heating relies on a slow gradient heat transfer from the heating mantle to the vial walls and finally into the reaction contents. Herein, CEM's Discover Prep™ was used to prepare chickpea protein vegan cream cheese for amino acid analysis. The Discover Prep (**Figure 1**.) is a microwave-based sample preparation system that utilizes precise temperature monitoring and compressed air cooling to rapidly prepare samples for amino acid analysis with accuracy and precision that matches traditional methods.

Figure 1. Discover Prep Single-Cavity Microwave System

Materials and Methods

All hydrolysis reagents and HPLC-grade eluents were obtained from commercial suppliers. The vegan cream cheese sample was purchased from a local grocery store and stored at 4 °C prior to analysis. Traditional amino acid hydrolysis was carried out in an air oven and compared to samples hydrolyzed in a Discover Prep microwave-based sample preparation system. All resulting hydrolysates were analyzed 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 for amino acid quantification. Asparagine (Asn) and glutamine (Gln) are converted into aspartic acid (Asp) and glutamic acid (Glu), respectively, under acid hydrolysis conditions; therefore, these amino acid residues are reported together.

Traditional Hydrolysis Method

50 mg homogenized vegan cream cheese was added to a glass vial, along with 2 mL of freshly prepared 6 N HCl with 1% (w/v) phenol solution. The reaction vial was then purged with N_2 and quickly sealed. This procedure was repeated until all samples were ready to be placed in a 110 °C oven for 20 hours. Upon completion, samples were removed from the oven and allowed to cool to room temperature prior to derivatization.

Discover Prep Acid Hydrolysis Method

A 100 mg sample of homogenized cream cheese was added to a pyrolyzed 35-mL Pyrex vial equipped with a Teflon® coated stir bar. A 5 mL aliquot of 6 N HCl containing 1% phenol (w/v) was then added to each microwave reaction vial. Vials were purged with N_2 for approximately one minute, quickly sealed with a Teflon-lined silicon cap, and placed in the autosampler rack for automated placement in the Discover Prep cavity. Lastly, a one-step Dynamic method was programed for the amino acid hydrolysis of vegan cream cheese.

Acid Hydrolysis:
Vial Type: Pyrex
Control Type: Dynamic
Temperature: 165 °C
Time: 30 min
Pressure: 300 PSI
Power: 300 W
Stirring: High

Sample Preparation for Analysis

Following traditional and Discover Prep microwave-assisted hydrolysis, all hydrolysates were neutralized with equimolar NaOH and then passed through 0.2 µm PTFE filters prior to derivatization with the Waters AccQ-Tag Ultra Derivatization kit. Briefly, 80 µL of borate buffer followed by 10 µL of the sample was added to a complete recovery vial, capped and vortexed. Next, 10 µL of the derivatization reagent was added to each sample vial, capped and vortexed. These prepared samples were then heated at 55 °C for 10 minutes prior to analysis.

Analysis

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 for the separation. The column temperature was at 55 °C, and the absorbance was monitored at 260 nm. The separation gradient used 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 to measure the concentration of each amino acid, Waters Amino Acid Standard (Waters Corporation, Part No. WAT088122) was derivatized at the final concentrations 1, 5, 10, 25, 50, 100, and 200 pmol/µL. Linear regression was also used to analyze the hydrolysates.[2] 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
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 and Discussion

The efficiency of the Discover Prep microwave for amino acid hydrolysis was evaluated by comparing the results obtained using this method to those from its traditional hydrolysis. Following LC-UV amino acid quantification, results compared very well to the traditional method. As depicted in **Figure 2**, recoveries for 17 proteinogenic and free amino acids all fell within a 75 to 120% recovery range with generally improved repeatability in comparison to traditional methods.

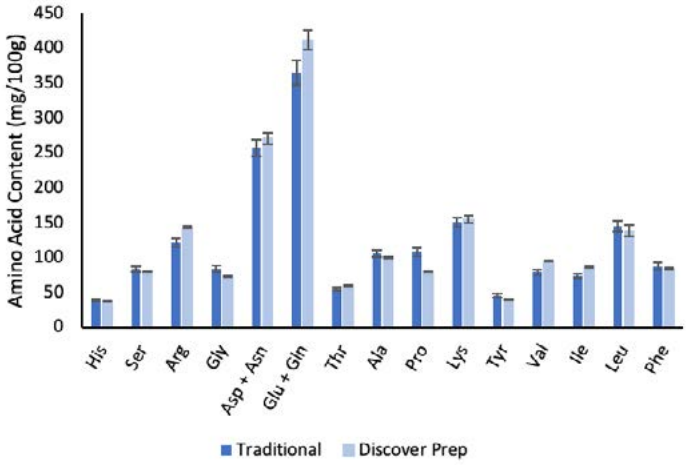

Figure 2. Vegan Cream Cheese Amino Acid Recoveries from Traditional and Microwave-Assisted Methods

The Discover Prep represents an efficient and a reliable alternative to time-consuming traditional methods for amino acid hydrolysis. The ability to prepare foodstuff samples for amino acid quantification in minutes compared to almost 24 hours opens the door for increased efficiency and opportunities for thorough method development. Parameters such as sample size, stirring speed, hydrolysis temperature and time can all be evaluated using the Discover Prep. Lastly, CEM's iWave® in situ temperature monitoring allows for rigorous reaction temperature control from day to day, an attractive feature that is not always expected with laboratory forced air ovens.

Conclusion

With the explosion of consumer interest in goods formulated with plant-based alternative proteins, manufacturers are focused on keeping up with the increased demand all while offering quality products. Further, with the implementation of new and more stringent regulations in this space, there is a need for robust, efficient, and reliable technologies to ensure these standards are met. One such area includes protein quality, an important feature that can be evaluated via amino acid analysis. Samples must first be prepared for this analysis by means of amino acid hydrolysis. CEM's Discover Prep microwave-based sample preparation system was successfully used to prepare a chickpea protein based vegan cream cheese sample for amino acid quantification. Prepared samples were analyzed via Waters ACQUITY UPLC-PDA detection and results compared very well to amino acid values obtained from the corresponding traditional chemistry sample preparation. All results were achieved in a more efficient manner, with other key benefits, such as ease of use and safer laboratory protocols, while maintaining result accuracy and precision.

References

[1] Hertzler, S.R.; Lieblein-Boff, J.C.; Weiler, M.; Allgeier, C. Plant Proteins: Assessing Their Nutritional Quality and Effects on Health and Physical Function. *Nutrients*. **2020**, *12* (12), 3704.

[2] Hong, P.; Johnson, D.; Trinite, D. A.; Warren, B.; Zhang, N. *Hydrolysis and Analysis of Amino Acids from Purified Peptides/ Proteins, Foods, and Feeds*; Waters Corporation, **2019**.

Find a Local Contact

cem.com/contact

