Using Microwave Energy for the Rapid Hydrolysis of Soy Flour for Amino Acid Analysis

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Abstract

The nutrition profile of food is critical for assessing its quality. An important macromolecule in food is its protein content. Protein content is typically assessed by measuring the amount of each individual amino acid. Quantifying each amino acid requires a hydrolysis step with acid or base to free the individual amino acids from proteins. The traditional approach to amino acid hydrolysis involves heating samples to 110 °C in sealed tubes for extended time periods up to 24 hours. Often, the subsequent analysis requires a derivatization step. In this work, the CEM Discover 2.0 microwave system was used to rapidly hydrolyze soy flour samples in the presence of acid or base. The samples were then neutralized, derivatized using the Waters AccQ-Tag Ultra reagent kit, and analyzed using a Waters ACQUITY UPLC H-Class system equipped with a PDA detector. The results for the soy flour samples showed that the Discover 2.0 yielded data with tight standard deviations for triplicate samples. The CEM Discover 2.0 is an ideal choice for laboratories seeking to reduce reaction times, combine instrumentation for acid and base hydrolysis, and obtain cleaner hydrolysates, while maintaining accuracy and precision.

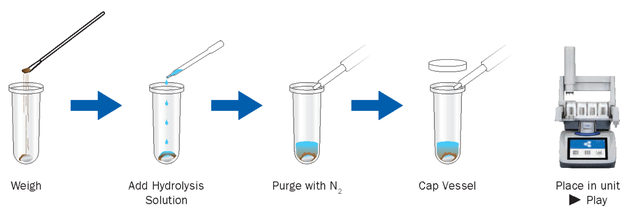
Discover 2.0 Hydrolysis

Acid

- Weigh 75 mg of soy flour into 35 mL Pyrex vessel with stir bar.
- Add 5 mL of 6 N HCl with 1% Phenol.
- Purge vessel with N_2 for 5 minutes.
- Seal vessel with a Teflon® lined cap.
- Place prepared vessels in the autosampler.
- Press Play.

Base for Tryptophan

- Weigh 300 mg of soy flour into a 35-mL TFM liner in a Pyrex vessel with a stir bar.
- Add 5 ml of 4 N NaOH.
- Purge vessel with N_2 for 5 minutes.
- Seal vessel with a Teflon® lined cap.
- Place prepared vessels in the autosampler.
- Press Play.



Discover 2.0 Parameters:

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

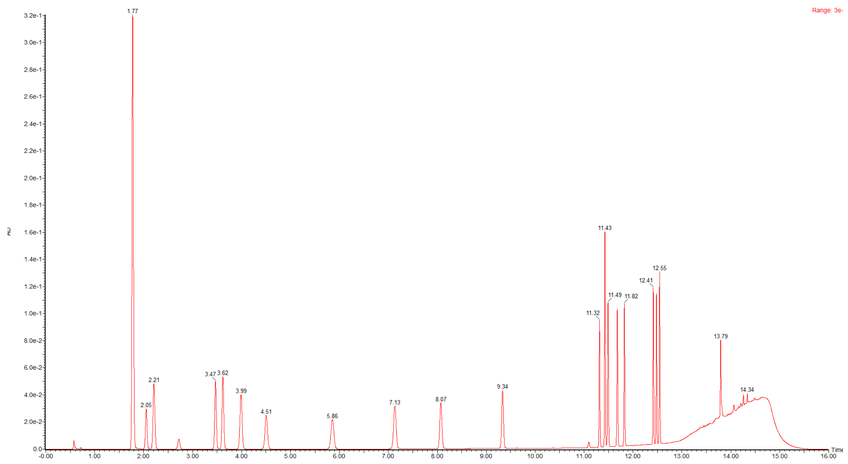
Base
Vessel Type: Pyrex
Control Type: Dynamic
Temperature: 195 °C
Time: 30 min
Pressure: 250 PSI
Power: 300 W
Stirring: High

Analysis

Post-hydrolysis pre-column derivatization was done using the Waters AccQ-Tag™ Ultra Derivatization kit. 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. Trp was analyzed separately using the same analysis method. Note: Cys and Met were not analyzed because the required preoxidation was not performed.

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
Trp	12.58

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

Results

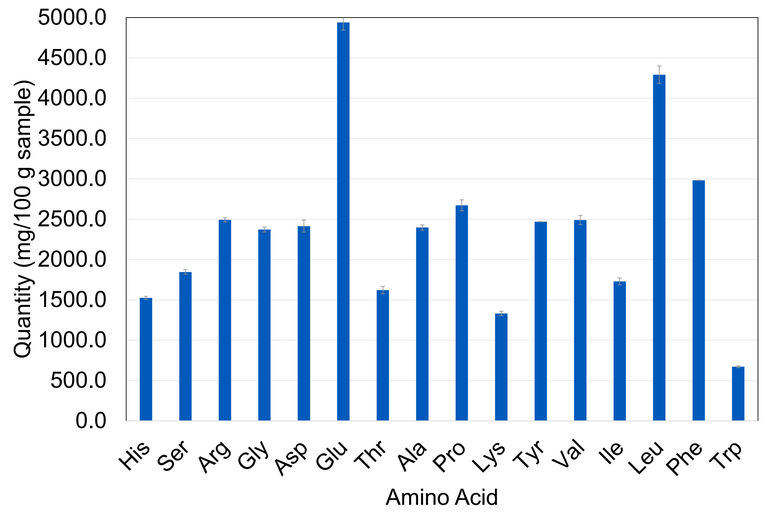
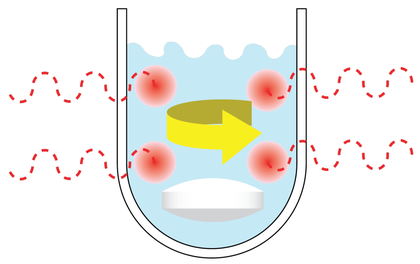


Chart 2: Amino acid data in triplicate with standard deviations obtained for soy flour material.

Key Benefits



- Comparable recoveries to traditional amino acid hydrolysis
- Outstanding accuracy and precision
- Reduced reaction times compared to traditional methods
- Both acid and base hydrolysis combined into one unit
- Cleaner hydrolysates
- Quicker sample prep time

References

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Dudley, G. B.; Richert, R.; Stiegman, A. E. *Chem. Sci.* **2015**, *6*, 2144-2152.
Chen, P.-K.; Rosana, M. R.; Dudley, G. B.; Stiegman, A. E. *J. Org. Chem.* **2014**, *79*, 7425-7436.
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**.