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Analysis of Amino Acids in Soy Flour Using the Chromaster HPLC System with Evaporative Light Scattering Detection

The amino acid composition of proteins can be determined by hydrolysis of the sample followed by HPLC analysis. Due to the poor spectral characteristics of most amino acids found in proteins, chromatography-based techniques used for amino acid analysis typically include a derivatization step either pre- or post- column. Presented here is a method that avoids a derivatization step through use of an evaporative light scattering detector (ELSD). The chromatographic separation is based on reverse-phase ion pairing, and detection with ELSD results in sample analysis within 50 minutes.

Experimental Conditions

Module	Conditions
Pump (5110)	Mobile Phase A: 20% CH_3CN, 0.025% TFA Mobile Phase B: 20% CH_3CN, 0.3% TFA Flow Rate: 1 mL/min Gradient: 0% B for 10 min, 0 – 80% B over 35 min., 0% B for 5 min.
Autosampler (5210)	Injection Volume: 40 µL
Oven (5310)	Temperature: 30 °C
Detector (SofTA 1300)	Spray Chamber Temperature: 25 °C Drift Tube Temperature: 60 °C
Column	Primesep 100A, 4.6 x 150 mm

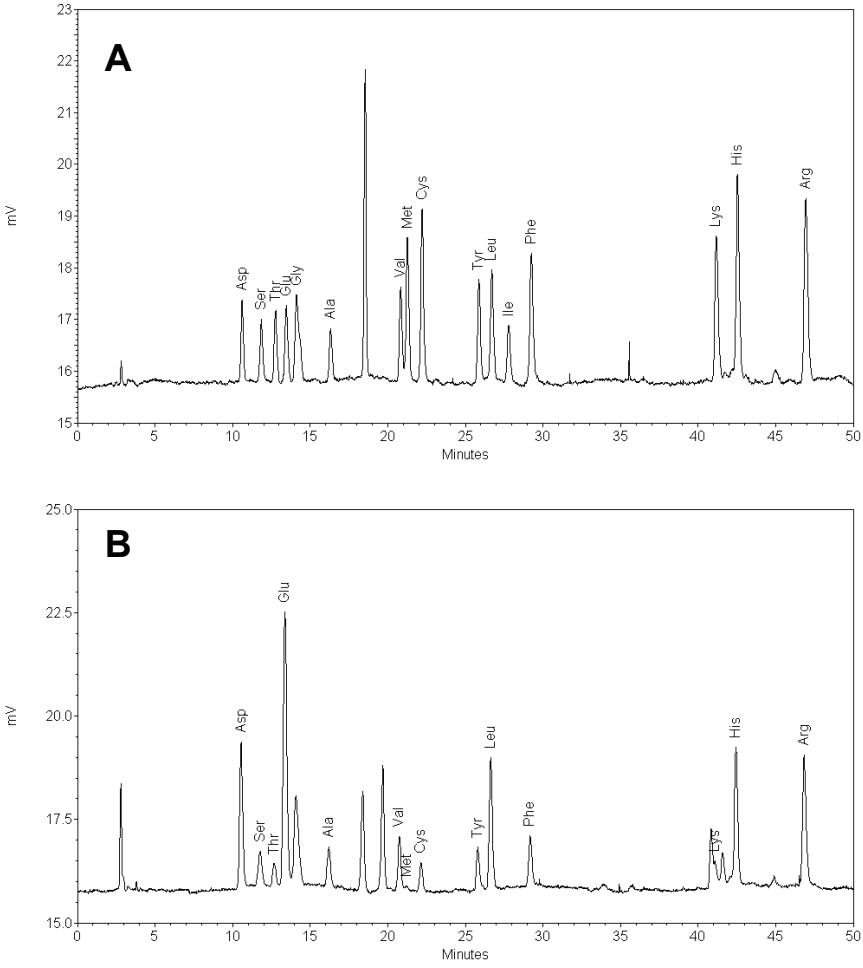
Results: Reproducibility and Linearity

Linearity (25 µM – 100 µM)	Average R^2 = 0.9903
Retention Time Reproducibility (N = 10)	Average RSD (%) = 0.09
Peak Area Reproducibility (N = 10)	Average RSD (%) = 6.2
Sensitivity (S/N) ≥ 3	Each amino acid: 1 nmole

Results – Chromatographs

A. Amino Acid Standard

B. Hydrolyzed Soy Flour



Discussion and Conclusion

Hitachi's Chromaster liquid chromatography system with ELSD effectively analyzes amino acids in soy flour. This method is capable of analyzing amino acids in hydrolyzed protein within 50 minutes.

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