

Rapid Screening of Amino Acids in Food by CE-ESI-MS

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Food Industry

Abstract

In the food industry, amino acids are measured to correlate flavor trends, monitor fermentation and assess the quality of the final product. Although amino acids are most commonly analyzed by HPLC with pre- or post column derivatization, capillary electrophoresis coupled to electrospray-mass spectrometry (CE-ESI-MS) is ideally suited for the rapid screening of free amino acids at low mg/L levels in complex matrices.

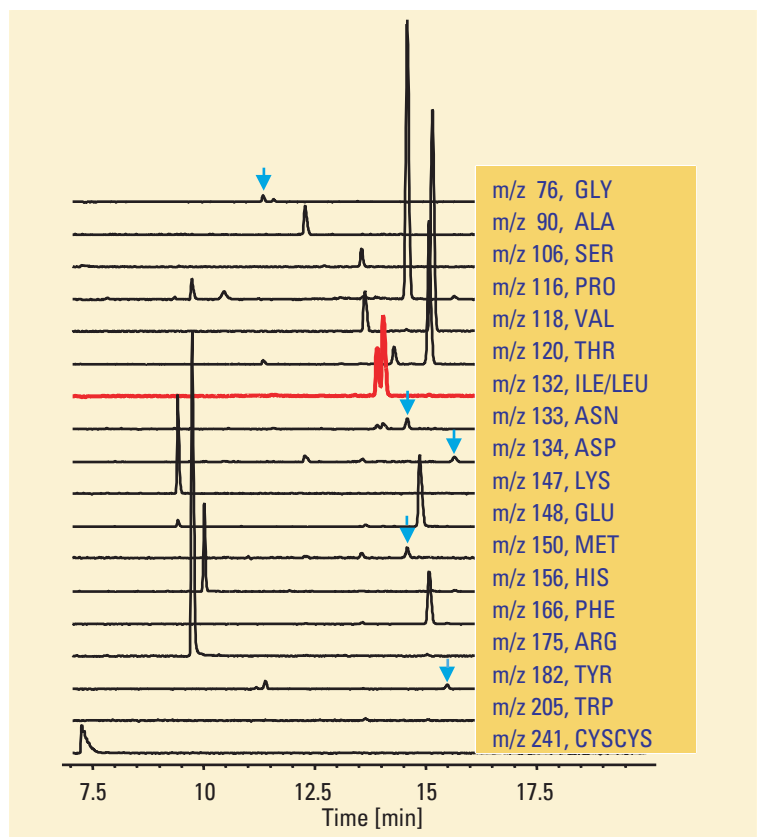


Figure 1
Amino acid analysis in soy sauce (selected ion traces).

Conditions

Sample

soy sauce, diluted 1:100 and ultrafiltered

Injection

3 sec @ 50 mbar

Capillary

bare fused silica
L= 100 cm, 50 μ m id

Buffer

1 M formic acid

Voltage

30 kV

Temperature

20°C

Preconditioning

4 min flush with run buffer at 1 bar

Sheath liquid

5 mM ammonium acetate in 50 % aqueous methanol, 10 μ L/min

Nebulizing gas nitrogen, 10 psi

Drying gas nitrogen, 10 L/min, 300 °C

Acquisition

positive mode, Vcap -4 kV,

fragmentor 70 V

Scan range

50–350 m/z



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Experimental

CE-ESI-MS analysis was performed using the Agilent Capillary Electrophoresis system with CE-MS capillary cassette coupled to the Agilent 1100 LC/MSD equipped with electrospray source and orthogonal sprayer for CE-MS. Agilent ChemStation software was used for instrument control. For method parameters see figure 1. A new capillary was flushed with run buffer for 20 minutes.

A 17-amino acid standard (2.5 mmol/L except Cys at 1.25 mmol/L in 0.1 N HCl, not containing Gln) was obtained from Pierce (Rockford, IL, USA). Asp and Trp were purchased from Wako (Osaka, Japan) and prepared at a concentration of 2.5 mmol/L in 0.1 N NaOH. Amino acid standards were mixed and diluted with deionized water to a final concentration of 250 $\mu\text{mol/L}$ (Cys 125 $\mu\text{mol/L}$).

Sample preparation was simple and consisted of dilution with deionized water and ultrafiltration through a 30 kDa cutoff filter to remove peptides and proteins.

Results

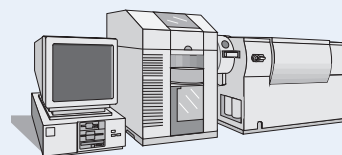
An optimized method for the rapid analysis of amino acids by CE-ESI-MS has been developed. Using an electrolyte with a pH value below the analyte's isoelectric point (< 2.77) allows the simultaneous determination of basic and acidic amino acids as positive molecular ions. In this study an ion at m/z 122 for Cys could not be observed. However, an ion at m/z 241 for Cys-Cys indicated oxidation by exposure to air.

When analyzing a standard composed of 19 amino acids, the assay was linear from 10–500 $\mu\text{mol/L}$. Detection limits ranged from 0.3 - 1.1 $\mu\text{mol/L}$ for basic amino acids to 6–11 $\mu\text{mol/L}$ for acidic amino acids. Migration time reproducibility was < 1.2 % RSD ($n = 8$), RSD for peak area was 2.0–4.7 % (except for Met, which gave decreasing peak areas due to oxidation).

The analysis of amino acids in food is important. Their monitoring can help track fermentation metabolites and is used to correlate flavor trends. Figure 1 shows the analysis of soy sauce by CE-ESI-MS. In addition to amino acids, soy sauce contains a great number of organic compounds with amino-functional groups. Using methods with derivatization can cause very complex separations. Without derivatization, as shown here, well-defined extracted ion traces can be obtained, allowing detection of amino acids in less than 13 minutes. Migration time reproducibility was < 0.4 % RSD ($n = 5$). Area reproducibility was 1.1–6.0 % RSD except for Met. This method was further applied to the analysis of amino acids in beer and sake.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent CE-MS Adapter Kit
- Agilent 1100 Series LC/MSD module with API Electrospray Source
- Agilent CE-ESI-MS Sprayer Kit
- Agilent ChemStation and CE-MS add-on software



References

- 1 Soga, T., Heiger, D., J. Anal. Chem. 2000, 72, 1236-1241.

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