DETERMINATION OF METHIONINE, THREONINE, LYSINE, AND CYSTINE IN FODDERS AND RAW MATERIALS BY CAPILLARY ELECTROPHORESIS

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INTRODUCTION

The method enables fast quantitative determination of amino acids methionine, threonine, lysine, and cystine in feeds, mixed fodders, and raw materials.

MEASUREMENT METHOD

Capillary electrophoresis method for the determination of amino acids is based on the differential migration of ionic forms of amino acids in a quartz capillary under the influence of the applied electric field. Identification of amino acids is performed basing on their own absorbance in the UV range at 190–200 nm. Capillary electrophoresis is performed twice: first for the determination of lysine, threonine, and cystine in borate buffer at 20 °C and then for methionine in borate buffer with β-cyclodextrin at 40 °C.
Hydrolysis of fodders is done according to the standard protocol in a close vessel with 6 M hydrochloric acid within 14–16 hours at 110 °C. Upon the removal of the excess of the acid, the sample solution is analyzed by capillary electrophoresis.

RANGES OF PERCENTAGE OF AMINO ACIDS

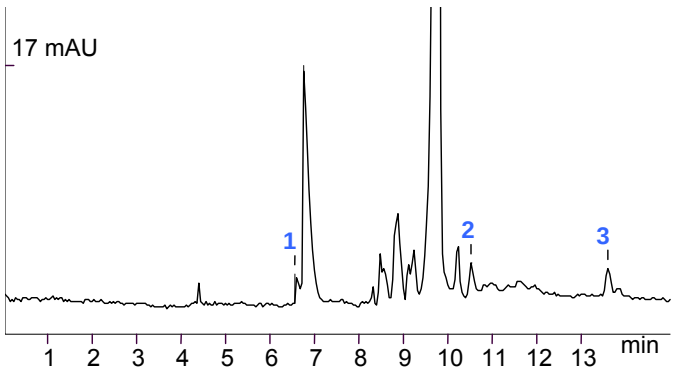
Amino acids	Percentage of amino acids, % (w/w)
Lysine	0.25–10.0
Threonine	0.25–3.0
Methionine	0.3–3.0
Cystine	0.2–2.0

EQUIPMENT AND REAGENTS

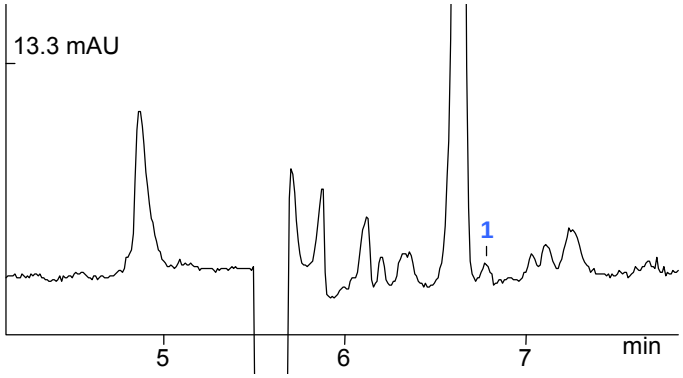
The “CAPEL®-105/105M” capillary electrophoresis system with a special capillary cassette for the amino acid analysis is used in measurements.
Data acquisition, collection, processing and output are performed using a personal computer running under “WINDOWS® 2000/XP” operating system with installed dedicated software package for acquisition and processing of chromatography data.
All reagents must be of analytical grade or higher.

EXAMPLES OF REAL ANALYSES

Buffer : borate (pH 9.2)
Capillary: L_{eff}/L_{tot} 65 / 75 cm; ID 50 µm
Injection: 450 mbar x sec
Voltage: + 20 kV
Temperature: + 20 °C
Detection: 190 nm
Sample: fish flour (100 mg)
Measurement results:
1 – lysine (3.12%)
2 – threonine (2.24%)
3 – cystine (0.56%)



Buffer: borate (ph 9.2) with β-cyclodextrin
Capillary: L_{eff}/L_{tot} 65 / 75 cm; ID 50 μm
Injection: 450 mbar x sec
Voltage: + 20 kV
Temperature: + 40 °C
Wavelength: 190 nm
Sample: fish flour (100 mg)
Measurement results:
1 – methionine (1.35%)



ADVANTAGES OF CAPILLARY ELECTROPHORESIS

Capillary electrophoresis has several advantages in comparison with the method of amino acid determination in the mixed fodders by amino acid analyser:

- absence of derivatization;
- low analysis cost;
- absence of an expensive chromatographic column;
- short analysis time.

LITERATURE

Komarova N. V., Kamentsev J. S., Solomonova A. P., and Anufrieva R. M. Determination of amino acids in fodders and raw materials using capillary zone electrophoresis // Journal of Chromatography B. – 2004. – V. 800. – No. 1–2. – P. 135–143.