

DETERMINATION OF AMINO ACIDS IN FODDERS AND RAW MATERIALS BY CAPILLARY ELECTROPHORESIS

INTRODUCTION

The method enables fast quantitative determination in feeds, mixed fodders, and raw materials of the following amino acids: arginine, lysine, tyrosine, phenylalanine, histidine, leucine and *iso*-leucine (total), methionine, valine, proline, alanine, glycine, cysteine, tryptophan, aspartic and glutamic acids. Since during the sample decomposition asparagine and glutamine are hydrolyzed to aspartic and glutamic acids, respectively, the content of these two acids represents the total content of both the acids and the amides.

MEASURING METHOD

The determination of amino acids in the samples is made either after preliminary alkaline hydrolysis for tryptophan or after acid hydrolysis for all the other amino acids. Free amino acids are transformed to phenyl thiocarbamyl derivatives (PTC derivatives) by means of phenyl isothiocyanate and their ionic forms are separated in the quartz capillary under the action of an electric field. The PTC derivatives are determined by measuring their own absorbance at 254 nm wavelength in a buffer solution.

CONCENTRATION RANGES

Ranges of measurable concentrations of amino acids are presented in the table below.

Amino acid (symbol)	Percentage of amino acid, % w/w *	Amino acid (symbol)	Percentage of amino acid, % w/w *
Arginine (Arg)	0.5–5.0	Threonine (Thr)	0.5–5.0
Lysine (Lys)	0.25–10.0	Serine (Ser)	0.25–5.0
Tyrosine (Tyr)	0.25–5.0	Alanine (Ala)	0.25–5.0
Phenylalanine (Phe)	0.25–5.0	Glycine (Gly)	0.25–10.0
Histidine (His)	0.5–5.0	Glutamic acid + glutamine (Glu+Gln)	0.5–10.0
Leucine+Isoleucine (Leu+Ile)	0.25–10.0	Aspartic acid + asparagine (Asp+Asn)	0.5–10.0
Methionine (Met)	0.25–5.0	Cysteine (Cys-Cys)	0.1–2.0
Valine (Val)	0.5–5.0	Tryptophan (Trp)	0.1–2.0
Proline (Pro)	0.25–10.0		

* For 100 mg sample

ADVANTAGES OF CAPILLARY ELECTROPHORESIS

Compared with amino acids determination in beer samples by amino acid analyzers, capillary electrophoresis (CE) has several advantages:

- Low analysis cost
- Absence of an expensive chromatographic column
- Short analysis time

EQUIPMENT AND REAGENTS

The following equipment and reagents are used for measurements:

- CAPEL®-105 Capillary electrophoresis system with a special capillary cassette for the amino acid analysis
- Distilled deionized water
- Sodium hydroxide
- Sodium tetraborate decahydrate
- Sodium carbonate decahydrate
- Disodium hydrogen phosphate dodecahydrate
- Sodium dihydrogen phosphate (mono- or dihydrate)
- Barium hydroxide octahydrate



- Sulphuric acid
- Hydrochloric acid, 37 wt. % in water
- Formic acid
- Hydrogen peroxide, 30 wt. % in water
- Ethanol, rectified
- 2-Propanol
- L-amino acids
- Phenyl isothiocyanate (PITC)
- β -cyclodextrin (β -CD)

All reagents must be of analytical grade or higher.

Data acquisition and integration is accomplished with PC with Windows® 95/98/NT/ME/2000/XP, using "Chrom&Spec® for Windows®" chromatographic software.

PREOPERATIONAL PROCEDURES

Preoperational procedures include: sampling and sample preparation, capillary conditioning, preparation of auxiliary and calibration solutions, and calibration of the CAPEL® Capillary Electrophoresis System.

MEASUREMENT PROCEDURE

Sampling is carried out in accordance with the standard certified protocol.

Capillary conditioning is performed by rinsing the capillary sequentially by 1 M hydrochloric acid, water, 1M sodium hydroxide, water and background electrolyte.

Measurements

The method prescribes the analysis of three portions of a sample. The analysis procedure for each of the portions differ by the sample pre-treatment, conditions of electrophoretic determination and the list of determined amino acids.

1. For the determination of arginine, lysine, tyrosine, phenylalanine, histidine, leucine and isoleucine, methionine, valine, proline, threonine, serine, Alanine, and glycine the acid hydrolysis of the mixed feed sample should be made using hydrochloric acid.

2. For the determination of aspartic acid, glutamic acid, and cysteine (in the form of cysteic acid) the portion is oxidized by performic acid with subsequent acid hydrolysis.

3. For the determination of tryptophan the alkaline hydrolysis of the sample portion by aqueous solution of barium hydroxide is made.

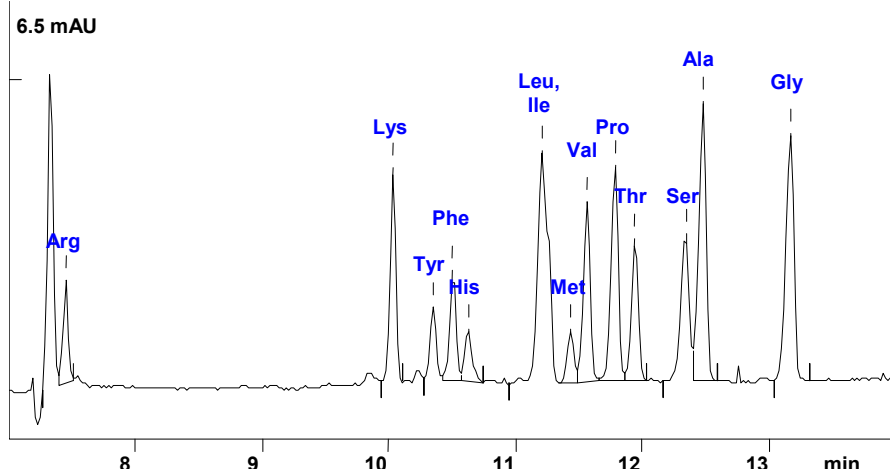
After removing the excess of acid (or alkali) free amino acids are transformed into the PTC derivatives by phenyl isothiocyanate in the presence of sodium carbonate. After that the solutions are analyzed by capillary electrophoresis.

DATA PROCESSING

Data analysis, integration and calculation of amino acids' concentrations are performed by the "Chrom&Spec® for Windows®" software.

EXAMPLES OF A REAL ANALYSIS

Sample yeast (analysis procedure №1)
Buffer: phosphate + β -cyclodextrin, pH=7.8
Capillary: $L_{\text{eff}}/L_{\text{tot}} = 65/75$ cm,
ID=50 μm
Sample injection: 150 mbar*sec
Voltage: + 25 kV
Temperature: + 30 °C
Pressure: 0 mbar
Detection: at 254 nm



This application note contains only basic reference data. To obtain a more detailed information please contact Lumex Ltd. at Moskovsky prospect 19, Saint-Petersburg, 190005, Russia. Tel +7 812 718-5390 Fax +7 812 316-6538.
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