DETERMINATION OF AMINO ACIDS IN BEER AND WORT BY CAPILLARY ELECTROPHORESIS

INTRODUCTION

Amino acids in beer originate mostly from malt. The following method enables fast quantitative determination in beer and wort of the following free 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

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.

CONCENRATION RANGES OF AMINO ACIDS

Concentration ranges for analyzed amino acids in beer samples are given in the table.

Amino acid	Standard value in beer, mg/l	Measurement range*, mg/l	Amino acid	Standard value in beer, mg/l	Measurement range*, mg/l
Ala	80–120	0.5–50	Lys	10–50	0.5–50
Arg	30–100	0.5–50	Met	0–10	0.4–40
Asn	0–10	1.0–40	Phe	10–80	1.0–50
Asp	20–50	0.5–50	Ser	10–30	0.3–40
Gln	0–10	0.5–40	Thr	5–15	0.5–40
Glu	30–50	1.0–50	Trp	1–20	1.0–40
Gly	20–50	0.2–50	Tyr	40–100	1.0–50
His	20–50	0.5–50	Val	50–100	0.4–50
Ile+Leu	10–100	0.5–50			

* sample 100 μl

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-105/105M CE system with a special capillary cassette for the amino acid analysis
- Distilled deionized water
- Sodium hydroxide
- Sodium tetraborate decahydrate
- Sodium carbonate decahydrate
- Sodium dihydrogen phosphate (mono- or dihydrate)
- Barium hydroxide octahydrate
- Disodium hydrogen phosphate dodecahydrate
- 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® 98/NT/ME/2000/XP, using Chrom&Spec® 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

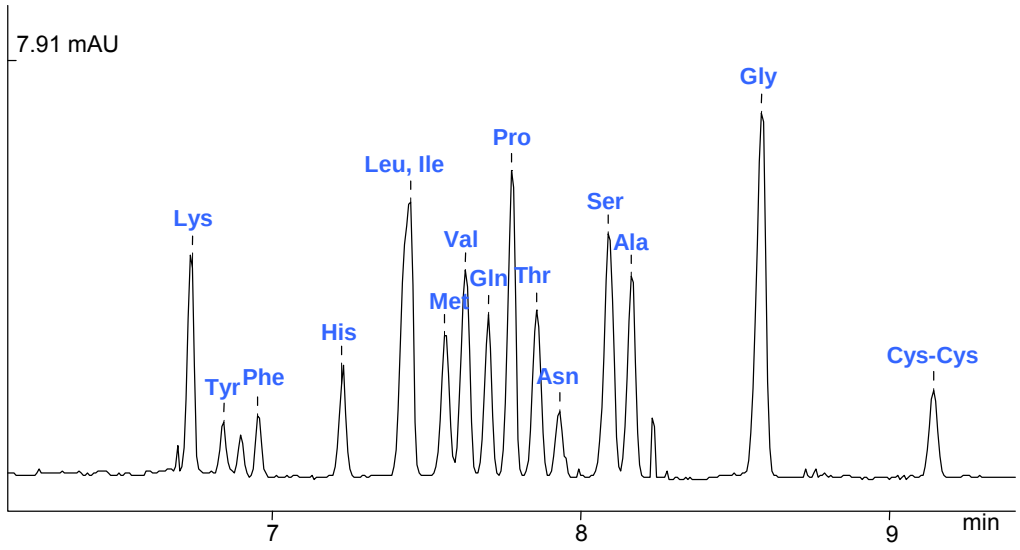
Sampling is accomplished in accordance with the standard certified protocol.
Capillary conditioning is done by flushing the capillary sequentially by 1M hydrochloric acid, water, 1M sodium hydroxide, water, and background electrolyte.

DATA PROCESSING

Data analysis, integration and calculation of tryptophane concentrations is done by the Chrom&Spec® software.

EXAMPLES OF REAL ANALYSIS

Sample: beer "X" (100 μl)
Buffer : phosphate with β-CD
Capillary: L_{eff}/L_{tot} 65 / 75 cm; ID 50 μm
Injection: hydrodynamic injection (5 s at 3000 Pa)
Voltage: +25 kV
Temperature: +30 °C
Wavelength: 254 nm



Other beer components, which can be determined by capillary electrophoresis method are as follows:

- Inorganic anions of water in brewing processes,
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- Organic acids (formic acid, acetic acid, and others),
- Hop and beer bitter acids (humulones and isohumulones),
- Vitamins (B group, ascorbic acid),
- Amines (histamine, volatile alkyl amines),
- Preservatives and color additives in finished products