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Determination of underivatized amino acids in beverage samples by capillary electrophoresis

Christian W. Klampfl[a,*], Wolfgang Buchberger[a], Martin Turner[a], James S. Fritz[b]

[a]*Department of Analytical Chemistry, Johannes-Kepler-University, Altenbergerstrasse 69, A-4040 Linz, Austria*
[b]*Department of Chemistry and Ames Laboratory, Iowa State University, Ames, IA 50011, USA*

Abstract

A method for the analysis of free amino acids in beverage samples by capillary electrophoresis and direct UV detection at 185 nm is presented. Separations were performed in a strongly acidic carrier electrolyte containing an alkanesulfonic acid and varying amounts of acetonitrile. The organic modifier permitted a manipulation of the separation selectivity for a number of analytes investigated in this study, thereby allowing design of appropriate carrier electrolyte compositions for a given analytical problem. Orange juices as well as beer samples could be analyzed with respect to their content of free amino acids. In this way, different types of beer could be distinguished by their amino acid patterns. © 1998 Elsevier Science B.V.

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1. Introduction

Free amino acids can be found in a number of biological tissues, body fluids, foods and medicines. Generally, analysis of these solutes is performed by chromatographic or electrophoretic techniques including a derivatization step mainly to enhance the sensitivity of spectroscopic detection. For this purpose a number of different derivatization agents suitable for UV or fluorescence detection have been described [1]. Since capillary electrophoresis (CE) became more and more important, this analytical method has also been employed for the separation and quantification of amino acids in various matrices [1,2].

Nevertheless a small number of papers have described the determination of amino acids by CE without a derivatization step [3–13]. Electrochemical detection [3–5], direct [6–9] and indirect UV detection [10,11], indirect fluorescence detection [12] and mass-spectrometric detection [13] have been employed for this purpose. Because of their zwitterionic character, these analytes can be separated in their cationic or their anionic form using a carrier electrolyte with the appropriate pH value. Most studies have described the analysis of these solutes using neutral or basic carrier electrolytes. Applications to real samples reported so far include peptide hydrolysis products [4], urine samples [5], infusion fluids [11] and fruit juices [7,8]. The latter two papers mainly deal with the analysis of fruit acids and three aromatic amino acids with respect to the adulteration of citrus juices.

Only recently we have demonstrated the separation of underivatized amino acids using an acidic carrier electrolyte containing alkylsulfonic acids and taking advantage of the UV absorptivity of these analytes at 185 nm for direct detection [9]. Compared to separations employing basic carrier electrolytes completely different migration orders for the

*Corresponding author.

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investigated solutes could be obtained. Although separations of standard solutions containing up to 20 different amino acids could be performed under acidic conditions, the suitability of this method for the analysis of real samples has not yet been tested.

Therefore, the aim of the present work was an investigation of the applicability of this analytical method in the field of food chemistry. Special emphasis was paid to the optimization of the carrier electrolytes for a particular analytical problem by addition of an organic modifier, in this case acetonitrile, in order to analyse the different amino acid patterns corresponding to the beverage samples included in this study.

2. Experimental

2.1. Instrumentation

The capillary zone electrophoresis instrument employed was a Quanta 4000 (Waters, Milford, MA, USA) equipped with a positive power supply and a fixed wavelength detector (mercury lamp) connected to a HP 3359 data acquisition system (Hewlett Packard, Palo Alto, CA, USA). Separations were carried out using fused-silica capillaries obtained from Polymicro Technologies (Phoenix, AZ, USA) with an effective length of 100 cm, an inner diameter of 50 μm and a detection window at a position of 8 cm from the end. Injection was performed hydrostatically at the anodic side by elevating the sample at 10 cm for a specified time. Direct UV detection at 185 nm was used.

2.2. Carrier electrolytes

The carrier electrolytes consisted of NaH_2PO_4, octanesulfonic acid and acetonitrile in varying concentrations. The pH value of these buffer solutions was adjusted with 2 *M* phosphoric acid prior to the addition of the organic solvent. Analytical grade reagents as well as high purity water obtained from a Milli-Q System (Millipore, Marlborough, MA, USA) were used throughout these experiments.

2.3. Standard solutions

Arginine (Arg), lysine (Lys), proline (Pro), tryptophan (Trp), phenylalanine (Phe), histidine (His), alanine (Ala), asparagine (Asn), serine (Ser), glycine (Gly), tyrosine (Tyr), cysteine (Cys) and glutamic acid (Glu) of highest purity available from Fluka (Buchs, Switzerland), Sigma (St. Louis, MO, U.S.A) and Merck (Darmstadt, Germany) were used for the preparation of standard solutions.

2.4. Samples and sample pretreatment

Beer samples were diluted 1:4, sonicated for 15 min to remove CO_2, spiked with Cys as internal standard for quantification and analyzed without further pretreatment. Fruit juices were filtered through a 0.45 μm disposable filter cartridge before injection.

3. Results and discussion

3.1. Optimization of the carrier electrolyte composition

As already mentioned above, strongly acidic carrier electrolytes containing alkylsulfonic acids have been found useful for the separation of underivatized amino acids. In a series of experiments we investigated the applicability of running buffers with a pH of 2.36 containing 10 m*M* NaH_2PO_4, 30 m*M* octanesulfonic acid and concentrations of acetonitrile ranging from 0 to 20% for the analysis of these solutes in beverage samples. Comparing separation selectivities of basic carrier electrolytes as employed by Lee and Lin [10] with the acidic electrolytes mentioned above, the following retention orders can be observed:

Basic conditions pH 11.0: Lys, Pro, Trp, Phe, His, Ala, Asn, Ser, Gly, Tyr, Cys and Glu.

Acidic conditions pH 2.36: Lys, Arg, His, Gly, Ala, Ser, Asn, Trp, Glu, Phe, Tyr, Pro and Cys.

Arg for example could only be analyzed using an acidic running buffer because of its comigration with

the electroosmotic flow (EOF) when a basic carrier electrolyte was used. Variations of the pH of the carrier electrolyte as well as type and concentration of the alkylsulfonic acid have already been discussed in detail in a previous paper mainly focusing on the separation mechanisms occurring when carrier electrolytes including high amounts of alkylsulfonates are employed [9]. Acetonitrile was used to manipulate the separation efficiency of this electrolyte, especially regarding adjacent peaks of analytes present in unfavorable concentration ratios. As can be seen in Fig. 1 the migration behavior of a number of amino acids is influenced by addition of an organic modifier to the running buffer. In particular, the resolution of Phe, Tyr and Glu turned out to be problematic without the presence of an organic modifier in the carrier electrolyte. At a level of 0% acetonitrile, the migration order achieved for these three solutes was Phe, Tyr and Glu, leading to only one peak for the pair Tyr and Glu. By addition of 5% of the organic modifier the migration order changed to Glu, Phe and Tyr and a complete resolution could be obtained for these three peaks. Raising the acetonitrile content up to 10% or more did not further improve the separation efficiency, but led to comigration of Phe and Glu as well as increased migration times. As can be seen from Fig. 1, migration times relative to Arg decreased while the concentration of the organic solvent was raised. The peak obtained for Arg was defined as reference because of the very slow EOF at the pH values used in this study leading to unfavorable times of analysis for EOF measurements.

3.2. Analysis of orange juices

The content of free amino acids was investigated in two different types of orange juice, a freshly prepared one and a canned one. Both samples were analyzed without any pretreatment except filtration. Regarding the amino acid pattern obtained for these samples, a 10 m*M* NaH_2PO_4 carrier electrolyte containing 30 m*M* octanesulfonic acid and 5% acetonitrile combined with direct UV detection at 185 nm was found to be the most suitable configuration for this analytical problem. Peak identification was performed by spiking the sample with the corresponding amino acids. As can be seen from Fig. 2 the electropherograms obtained for these two orange juices showed significant differences. The amount of Pro found in the canned orange juice was 550 ppm, the highest concentration of a free amino

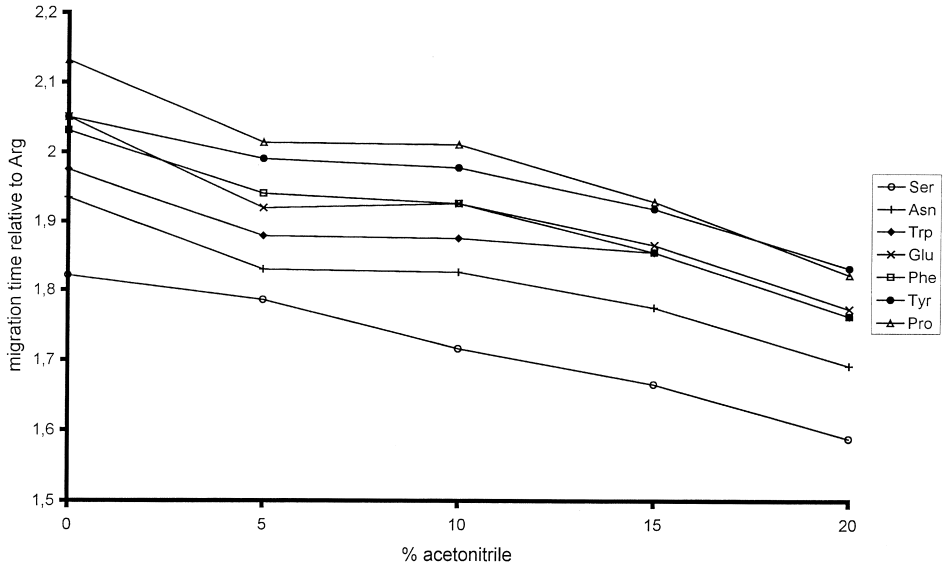

Fig. 1. Influence of the acetonitrile content of the carrier electrolyte on the migration behavior of selected amino acids relative to arginine. Carrier electrolyte: 10 m*M* NaH_2PO_4 containing 30 m*M* octanesulfonic acid, pH 2.36. Applied voltage: 30 kV.

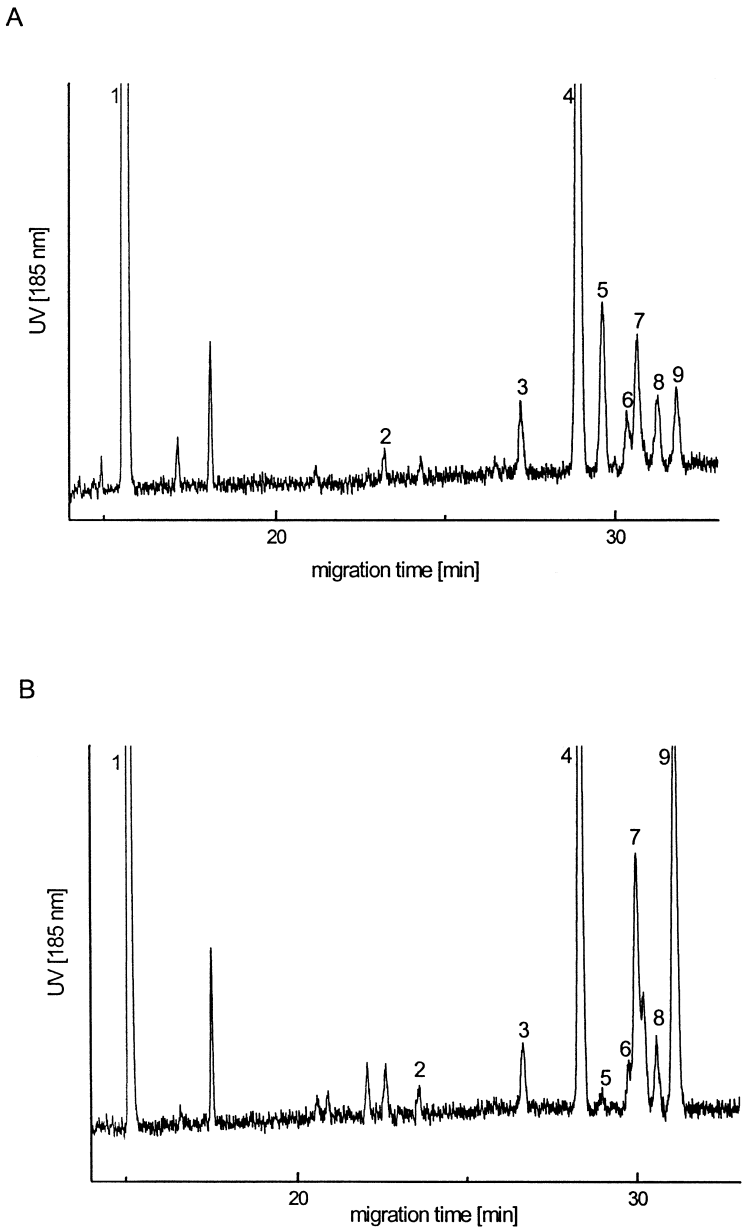

Fig. 2. Electropherograms of a freshly prepared (A) and a canned orange juice (B). Carrier electrolyte: 10 m*M* NaH_2PO_4 containing 30 m*M* octanesulfonic acid and 5% acetonitrile, pH 2.36. Applied voltage: 30 kV. Injection time: 15 s. Detection: direct UV at 185 nm. Peaks: 1=arginine; 2=alanine; 3=serine; 4=asparagine; 5=tryptophan; 6=glutamic acid; 7=phenylalanine; 8=tyrosine; 9=proline.

acid occurring in this study. Looking at the freshly prepared juice, only one-quarter of this quantity could be detected for Pro. Notable differences between the two investigated samples could also be found for Trp, whereas Arg and Asn could be identified as main components in both juices.

3.3. Analysis of beer samples

Free amino acids can also be found in beer beside proteins and peptides, mainly originating from the malt component of this alcoholic beverage [14]. For this reason a number of different types of beer including a nonalcoholic-beer, a white-beer, a Chinese rice-beer, a stout and a Lager-beer were ana-

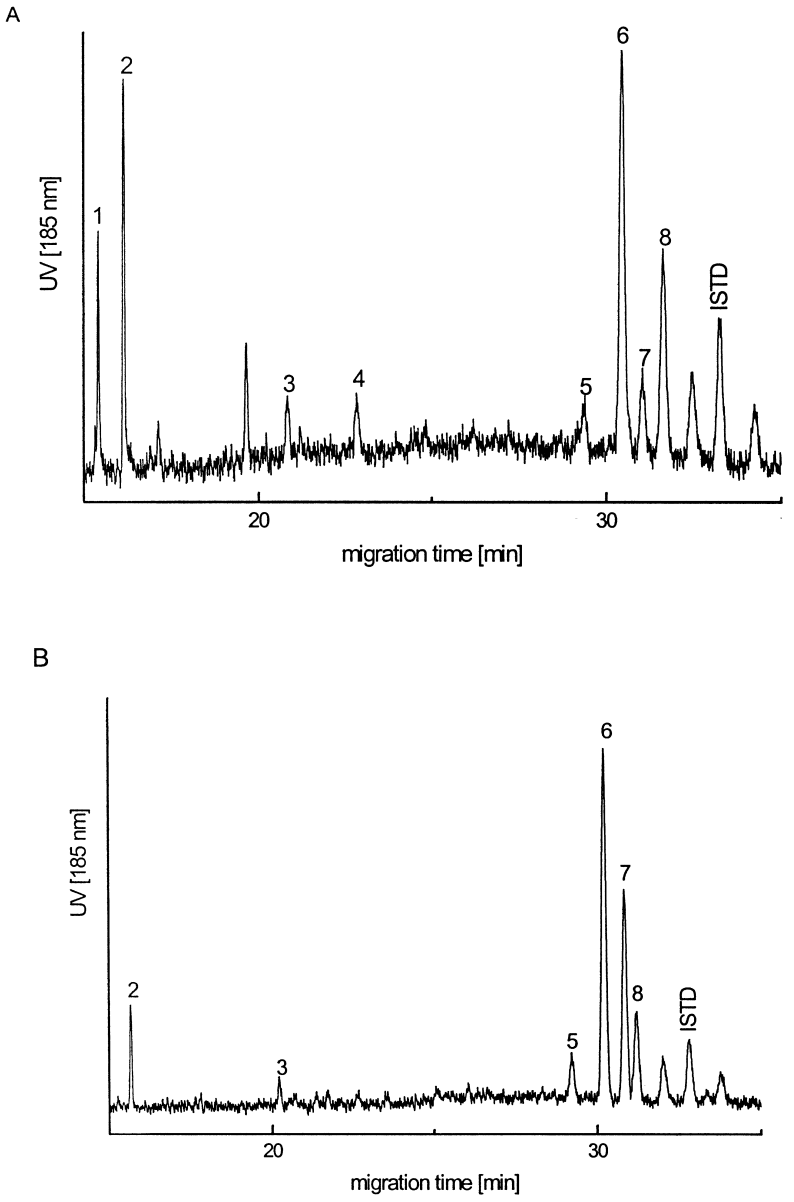

Fig. 3. Electropherograms of a rice-beer (A) and a white-beer sample (B). Carrier electrolyte: 10 m*M* NaH_2PO_4 containing 30 m*M* octanesulfonic acid, pH 2.36. Applied voltage: 30 kV. Injection time: 15 s. Detection: direct UV at 185 nm. Peaks: 1=lysine; 2=arginine; 3=glycine; 4=alanine; 5=tryptophan; 6=phenylalanine; 7=tyrosine; 8=proline.

lyzed using a 10 m*M* NaH_2PO_4 carrier electrolyte (pH 2.36) containing 30 m*M* octanesulfonic acid. The addition of acetonitrile, as used in the analysis of orange juices, proved to be less suitable for this analytical problem, as the separation of the amino acids detected in these samples could not be enhanced by addition of the organic solvent. In Fig. 3 the electropherograms obtained for two types of beer, a Chinese rice-beer and a white-beer are depicted. Significantly different amino acid patterns were obtained for these two samples. In rice-beer eight peaks could be identified, while in the white-beer sample only six peaks could be clearly assigned. Quantification of the analytes was performed by external calibration using standard mixtures covering the concentration range of interest with respect to the investigated sample. Calibration plots were found to be linear between 1000 and 50 to 5 mg/l depending on the molar absorptivity of the corresponding solute. To prevent errors related to the injection procedure all quantitative results were corrected using Cys as internal standard. The limits of detection (LODs) as well as standard deviations were found to be strongly depended on the spectroscopic properties of the corresponding analyte. Relative standard deviations obtained for standard mixtures of the solutes at concentration levels comparable to the samples were less than 2% for Trp (an amino acid exhibiting strong UV absorbance at the detection wavelength) and 3.5 to 9% for aliphatic amino acids showing only weak UV absorbance. LODs were 0.5 ppm for Trp and Phe and 10–50 ppm for the other analytes. As can be seen from Table 1, different amino acid ratios were achieved for the beer samples under investigation. The concentrations listed were the average of three consecutive runs. Standard deviations obtained for analytes present in concentrations near the LOD were less favorable as can be seen from this table. Regarding these results it can be deduced that the stout and the rice-beer contained a higher total content of free amino acids than the other beers. Pro was found in all analyzed samples in concentrations higher than 200 ppm. The same can be stated for Tyr except for the rice-beer. Trp, Phe, Gly and Arg could also be detected in all analyzed samples but the concentrations obtained for these analytes were somewhat lower. On the other hand Ser was only present in one investigated sample, the nonalcoholic-beer, and His just in the nonalcoholic- and the Lager-beer.

4. Conclusions

The results obtained in this work indicate that underivatized amino acids can be analyzed in beverage samples by CE with direct UV detection using the appropriate carrier electrolyte system. Main advantages are the simplicity and the rapidness of this method, allowing a fast screening of the amino acid patterns for a number of different types of samples in food chemistry. No sample pretreatment as well as no derivatization steps prior to analysis are necessary. But it has to be stated, that the LODs are relatively high for many of the amino acids. Solutes with strongly UV absorbing molecular sites like Phe or Trp can be detected in low ppm concentrations, but the LOD for the aliphatic amino acids was in the range of 10 to 50 ppm. Except these detection limitations, the method presented in this work offers

Table 1
Comparison of the content of amino acids (mg/l) in different types of beer

Lysine	282±1.2	n.d.	588±3.3	246±6.5	124±12.8
Histidine	30±7.2	n.d.	n.d.	n.d.	31±6.5
Arginine	91±2.4	76±2.3	120±5.5	165±1.5	42±7.8
Glycine	109±6.4	138±8.3	140±15.9	242±3.0	68±4.7
Alanine	n.d.	n.d.	193±8.3	190±3.0	110±12.6
Serine	127±27.5	n.d.	n.d.	n.d.	n.d.
Tryptophane	48±7.7	49±1.0	25±2.3	63±4.5	36±11.1
Phenylalanine	94±0.4	95±1.4	55±2.6	144±2.0	58±7.5
Tyrosine	252±1.6	419±2.2	71±2.6	492±4.9	215±5.8
Proline	246±3.4	546±1.3	620±1.6	576±7.0	296±7.6

n.d. = not detected; values represent means (±relative standard deviation %) of three determinations.

a fast and easy quantitative determination of amino acids in a variety of matrices.

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