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Determination of reducing sugars in selected beverages by capillary electrophoresis

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Abstract A method for the analysis of reducing sugar enantiomers in beverages using capillary zone electrophoresis is presented. Based on previous results, a resolution of all aldo-hexoses, -pentoses, -tetroses and trioses is achieved. Additional separation of uronic acids, deoxy and amino sugars in different buffer systems is demonstrated. Derivatives of sugar enantiomers change their migration order if derivatized with the phenylethylamine enantiomer. Thus, the use of a chiral derivatizing agent leads to simpler peak validation and opens new opportunities for the development of new applications. Screening of pharmaceutical drugs or food for rare sugar enantiomers with a detection limit of 25 fmol (5 μM) is feasible. Adaptation of the general method to wine, juice and instant coffee is demonstrated. The aldose, uronic acid and deoxy aldose enantiomer composition of the presented beverages is obtained in a single run.

Key words Capillary electrophoresis · Carbohydrates · Enantiomers · D/L-Sugars

Introduction

As generally accepted, sugars may serve as indicators for food authenticity, processing or adulteration. Especially minor components or rare sugar enantiomers are of analytical interest. The data available suggest that systematic studies on sugar enantiomer composition in nature are still far from being comprehensive. Therefore, we believe that an efficient analytical technique [1, 2] for the separation of sugar enantiomers is of considerable importance in biological research and food analysis.

Some carbohydrates, such as xylose, glucose and fructose, have been recognized as good tracers for instant coffee adulteration [3]. In wine there are highly increased amounts of arabinose, rhamnose, ribose, xylose and galactose after enzyme treatment. Some of them are not detectable in the absence of enzymatic processing [4]. The same effect of increasing pentose and galacturonic acid amounts is evident in *Botrytis cinerea* infected wine.

In the analysis of pharmaceutical drugs, capillary electrophoresis (CE) methods are well established [5–9]. The application of CE in food analysis mainly comprises the identification of proteins [10–13] to detect heat processing or adulteration of milk and dairy products [14–16]. In general, CE carbohydrate food analysis is not yet widespread. Although there are lots of different CE techniques for carbohydrates [5], only a few applications [17, 18] for juice [19] and hydrolysed polysaccharides [20] are described. Most methods did not determine the enantiomeric composition of the sugar mixtures.

In the following, we will introduce a technique for food carbohydrate enantiomer analysis.

Materials and methods

Capillary electrophoresis. All separations were performed using a P/ACE 2210 CE system with UV detector and a P/ACE 5500 with diode-array detector (Beckman Instruments, Munich). Photometric on-column detection was carried out at 200 nm and 'System Gold' software was applied for data acquisition and analysis. For all separations, uncoated fused-silica capillaries (Beckman eCap) of 50 μm i.d. (385 μm o.d.) were used. The capillary length was 77 cm and the detector was situated 7 cm from the cathodic end (normal polarity). The capillary was flushed with 0.1 M NaOH for 3 min and with buffer for a further 3 min prior to each analysis. Samples were injected by pressure (3.45 kPa) and separation was carried out at ambient temperature (Beckman capillary cartridge coolant).

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Chemicals and buffers. All monosaccharide standards were purchased from Sigma, Aldrich and Janssen Chimica. Sodium cyanoborohydride ($NaBH_3CN$) was a product of Aldrich. A solution of 9 mg of the reducing agent sodium cyanoborohydride in 30 µl of water was prepared.

The reagents used for the reductive amination, *R*-(+)-1-phenylethylamine (*R*-PEA) and *S*-(–)-1-phenylethylamine (*S*-PEA) were obtained from Merck. The amines were dissolved in water (1 M solutions) and the pH was adjusted to 6.5 with 10 M HCl.

Operating buffer solutions of the desired concentration were prepared by dissolving appropriate amounts of sodium tetraborate in water and adjusting the pH with 10 M NaOH or 10 M HCl. If necessary, acetonitrile was added as organic modifier.

Sample preparation. Generally, sample preparation was unnecessary. To detect traces of rare sugars, we evaporated 1 ml of wine or juice under mild conditions to dryness and redissolved the residue in 50 µl of water. Instant coffee powder was dissolved in water. All aqueous samples were stored at –20 °C until use.

Reductive amination. 12 µl of amine solution was added to 10 µl of the sample solution. This reaction mixture was kept at 90 °C for 10 min. The resulting Schiff base was treated with 4.5 µl sodium cyanoborohydride solution at 90 °C for 1 h to form a stable amine. After addition of 100 µl water the reaction mixture was stored at –20 °C. Prior to introduction, these samples were further diluted.

Results and discussion

After optimization of capillary length, temperature and applied voltage our capillary zone electrophoresis (CZE) method [1] separated 28 aldose derivatives in about 45 min (Fig. 1). Separation was based on the well-known formation of negatively charged complexes of the sugar hydroxy groups with tetrahydroxyborate [21] after derivatization with a chiral UV tag.

Reductive amination of aldoses with *S*-PEA proves to be advantageous for the following reasons:

1. The derivatives exhibited enhanced UV absorption. The detection limit corresponded to 25 fmol (5 µM) for an aldose derivative. The relationship between peak area and the amount of standard monosaccharides present was found to be linear over three orders of magnitude (10^{-10}–10^{-13} mol), with a satisfactory correlation coefficient ($R^2 = 0.998$).

2. Reductive amination reduced the number of possible isomers of individual sugars in the solution to the open–chain form. This unified the complexation of sugar hydroxy groups with tetrahydroxyborate ions and sharpened the resulting peak shape.

3. Derivatization of aldose enantiomers with the chiral amine resulted in pairs of diastereomer derivatives which could be separated in an achiral environment.

To control the results, samples were derivatized with the other phenylethylamine enantiomer. Thus, L- and D-glucose switched their positions in an electropherogram, upon derivatization with the enantiomeric amine. Figure 2 shows the change in migration order and the chance of enhanced resolution (for D-glc and D-man) by selecting the adequate amine enantiomer.

Adaptation of this method [1, 2] to special requirements, e.g. faster separation of less complex sugar mix-

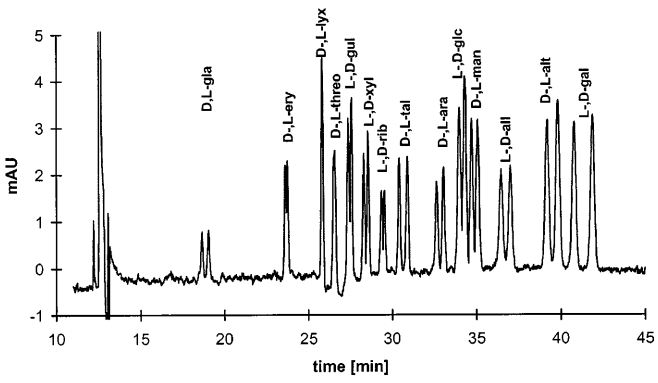

Fig. 1 Electropherogram of 28 aldose derivatives (conditions: constant voltage 28 kV, capillary length 77/70 cm, i.d. 50 µm, 50 mM borate buffer +30% acetonitrile, pH 10.3, temperature 20 °C, detection 200 nm)

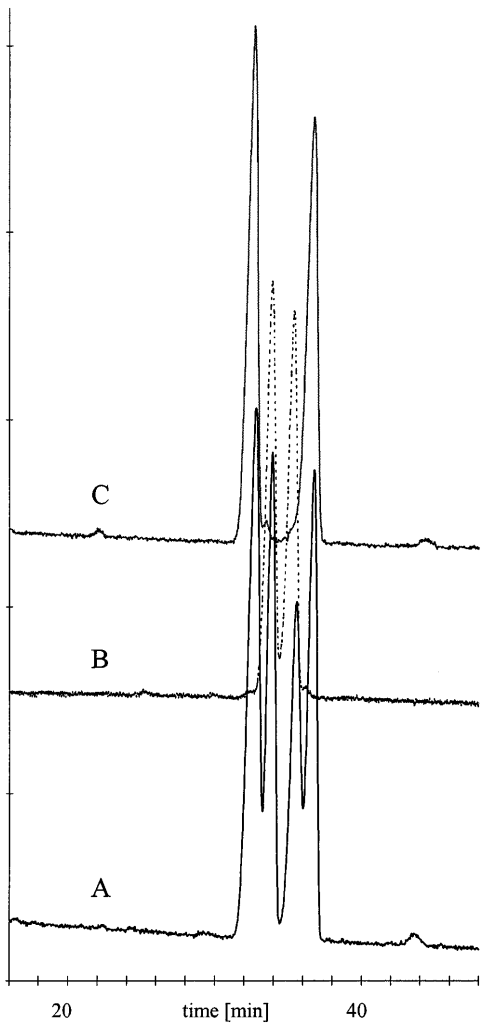

Fig. 2 Electropherogram of the derivatives of *A* L/D-glc and D/L-man with *S*-PEA, *B* D-glc and D-man with *S*-PEA, *C* D-glc and D-man with *R*-PEA (in the order of migration)

tures, succeeded better by adjusting the buffer pH and borate concentration or the amount of the organic modifier.

Analysis of white wine

Based on these results, a method for the estimation of the sugar composition of wine was established. Pentoses are found in grape juices in complex polysaccharides. During fermentation, cleavage of these polysaccharides releases the pentoses, which cannot be fermented by yeast. The amount of L-arabinose can especially increase dramatically [3]. Additionally, an infection with *B. cinerea* or enzyme treatment can also lead to higher amounts of L-arabinose, galacturonic acid and other sugar components [4].

To illustrate the suitability and separation efficiency, we prepared a standard comprising a typical sugar composition [4] of an enzyme-treated wine (Fig. 3). Derivatives of L-rhamnose, D-xylose, D-ribose, D-glucose, L-arabinose, D-galactose and D-galacturonic acid were separated in a single run. Amino alditols of pentoses, hexoses and deoxy sugars were easily detected within less than 15 min. According to the electrophoretic principle, simultaneous determination of the negatively charged uronic acid and the neutral aldose derivatives lengthened the time for analysis.

Separation of a derivatized white wine is demonstrated in Fig. 4. To achieve a better D-glucose/D-mannose separation the sample was derivatized with R-PEA according to Fig. 2. In the presence of D-glucose, estimation of D-mannose, L-arabinose, D-galactose and D-galacturonic acid was possible. Traces of D-xylose and D-ribose were detected. Other sugars were found only in negligible amounts.

Comparative analysis of two similar Riesling wines of different vintages (Fig. 5) led to almost identical

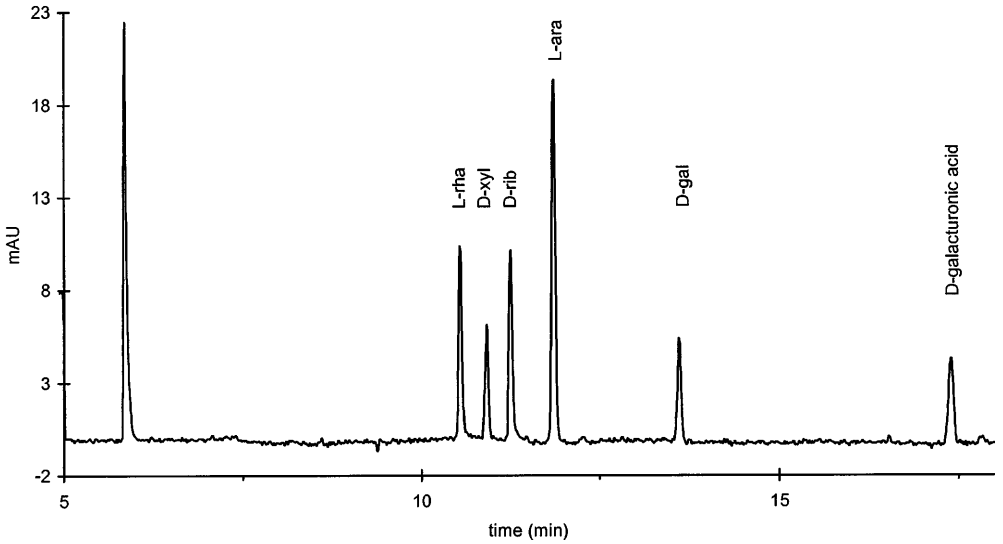

Fig. 3 Electropherogram of a derivatized standard of an enzyme-treated white wine (conditions: constant voltage 28 kV, capillary length 77/70 cm, i.d. 50 μm, 50 mM borate buffer, pH 8.8, temperature 22 °C, detection 200 nm)

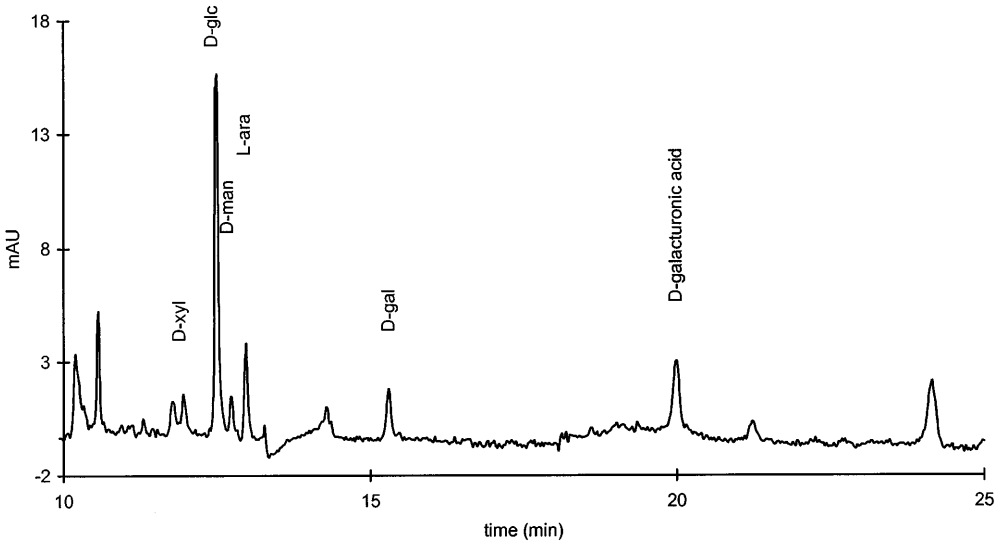

Fig. 4 Electropherogram of a derivatized Muller Thurgau wine (conditions: constant voltage 28 kV, capillary length 77/70 cm, i.d. 50 μm, 50 mM borate buffer, pH 8.8, temperature 20 °C, detection 200 nm)

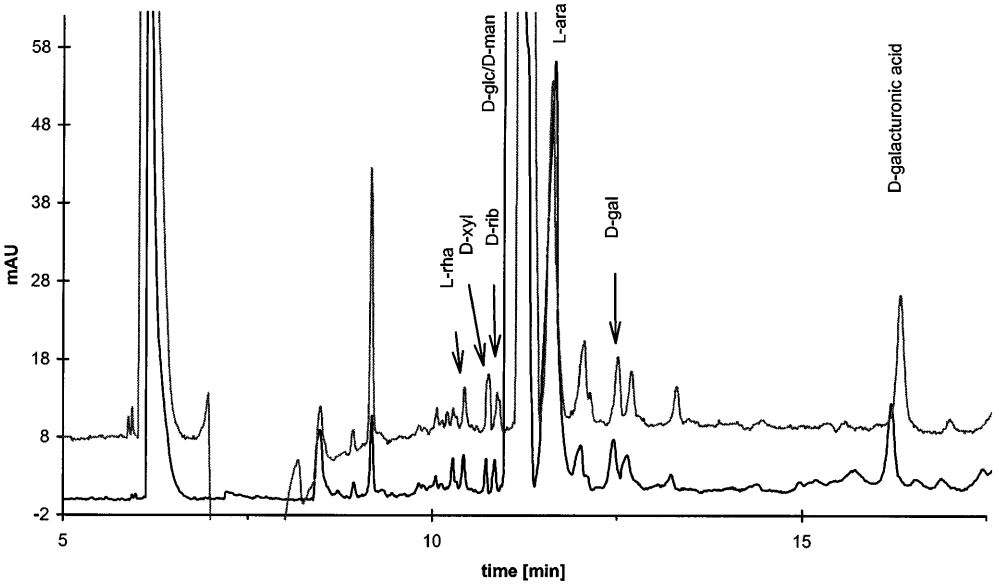

Fig. 5 Electropherograms of derivatized Riesling wines from different vintages

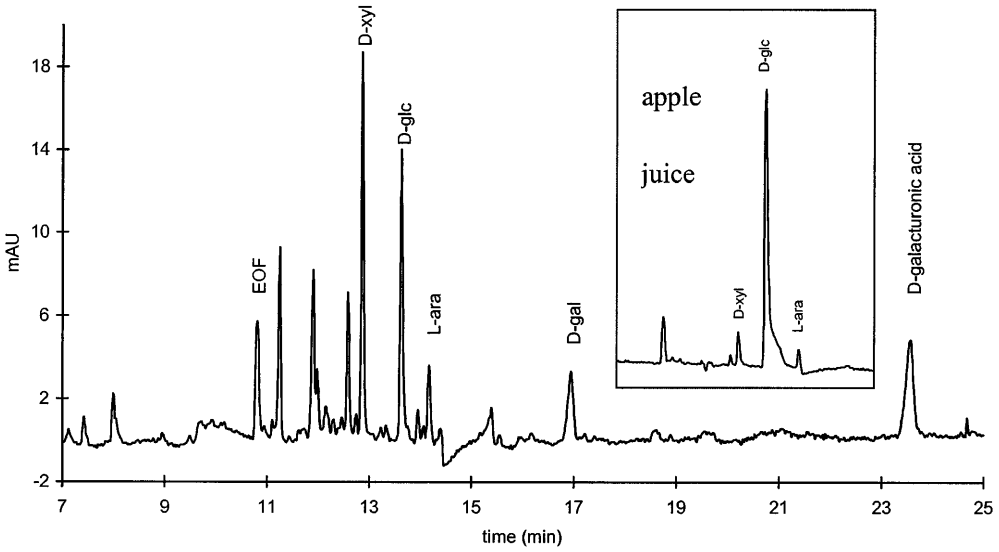

Fig. 6 Electropherogram of derivatized apple wine (apple juice) (conditions: constant voltage 28 kV, capillary length 77/70 cm, i.d. 50 μm, 50 mM borate buffer, pH 8.8, temperature 22 °C, detection 200 nm)

electropherograms. Only significant differences of the arabinose/galacturonic acid ratio were found. Here the ratio varies from 2.5 to 6.

Analysis of apple juice and wine

The same effect of sugar release from polysaccharides during fermentation was demonstrated in the electropherograms of derivatized apple juice and – the Hessian regional speciality – apple wine. As expected, apple juice mainly contains D-glucose and only small amounts of pentoses (Fig. 6). Fermentation results in a decreasing glucose part and increasing D-xylose, L-arabinose, D-galactose and D-galacturonic acid values. The pattern of additional unidentified substances may serve as a 'fingerprint' for different kinds of apples or brands.

Analysis of instant coffee

An electropherogram of derivatized instant coffee is shown in Fig. 7. The separation of this complex sample reveals the efficiency of the presented CE method. Traces of D-xylose were easily identified. As already mentioned, this pentose is recognized as a good tracer for adulteration with coffee husk or parchment.

It is not acceptable to draw final conclusions on adulterations from such isolated wine, juice and coffee samples without a comprehensive data basis [4]. Because of the low detection limit of the method, further experiments are required to distinguish between natural biological variation and adulteration. So we confine ourselves at the present stage only to the demonstration of the analytical capacity of our CE method.

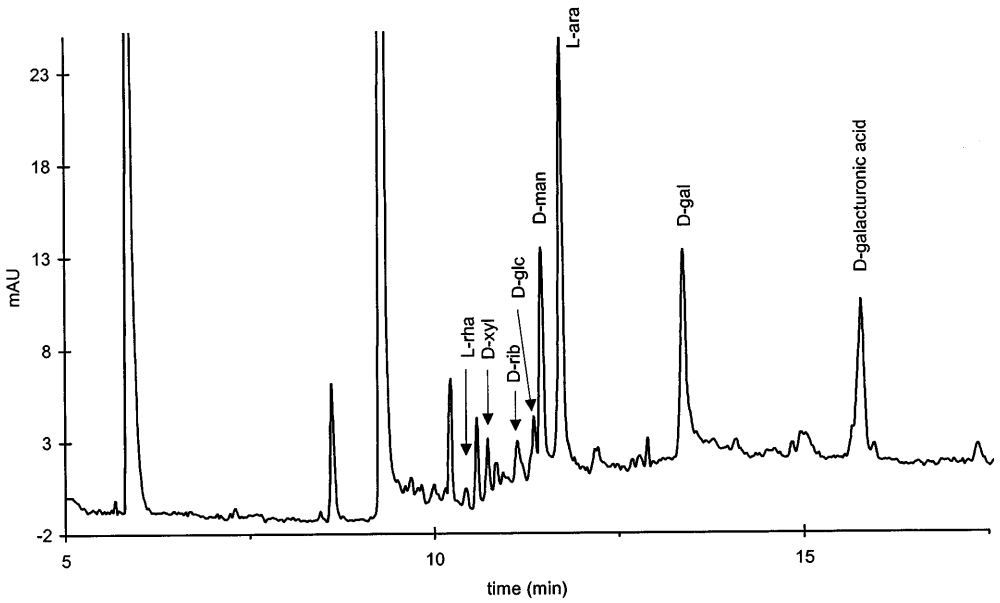

Fig. 7 Electropherogram of a derivatized instant coffee (conditions: constant voltage 28 kV, capillary length 77/70 cm, i.d. 50 μm, 50 mM borate buffer, pH 8.8, temperature 22 °C, detection 200 nm)

Conclusions

The CZE method presented allows easy and efficient analysis of sugar enantiomers in beverages after derivatization. Our examples demonstrated that CZE may serve as an excellent tool for the determination of food adulteration. The total aldose, uronic acid and deoxy aldose enantiomer composition of the presented beverages is accessible in a single analytical run.

In comparison to HPLC methods [3], CE analysis is much less time consuming. Determination of different aldose enantiomers after derivatization is feasible. Usually there is no need for a sample clean-up process. Only for the precise determination of minute amounts of minor sugar components is mild concentration recommended.

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