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Determination of propionate in bread using capillary zone electrophoresis

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ABSTRACT

A method for the determination of propionate in bread is described. The propionate was extracted from the bread with a repeated extraction procedure and measured using capillary zone electrophoresis in the indirect UV mode applying a background electrolyte of 0.005 *M* Tris adjusted at pH 4.6 by adding benzoic acid. Using laboratory-baked bread containing known amounts of sodium propionate, recoveries of *ca.* 95% could be established, validating the method.

INTRODUCTION

In the preparation of bread, propionate is often applied because of its anti-bacterial properties. According to the Dutch Food and Drugs Act, maximum values are prescribed with respect to the presence of propionate in bread, hence there is a need for its determination. Gas chromatography (GC), high-performance liquid chromatography (HPLC) and ion chromatography (IC) can be applied for this purpose. A disadvantage of GC is that derivatization is necessary and in HPLC and IC matrix effects can be troublesome. As capillary zone electrophoresis (CZE) is suitable for the determination of ionic species in a complex matrix [1–3], we studied the ability of CZE for the determination of propionate in bread. Special attention was paid to the identification of propionate and a simple extraction procedure for isolating propionate from a bread matrix. As the preparation of calibration graphs is time consuming, we further studied the possibility of repeated injections of several standard sample solutions within one electrophoretic run. The determination of propionate in bread and the recoveries with the extraction procedures were evaluated using laboratory-baked bread containing different known amounts of propionate.

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EXPERIMENTAL

Instrumentation

For all experiments a P/ACE System 2000 HPCE instrument (Beckman, Palo Alto, CA, USA) was used. All experiments were carried out with Beckman eCAP capillary tubing (75 μm I.D.) with total length 46.7 cm and a distance between injection and detection of 40.0 cm. The wavelength of the UV detector was set at 214 nm. All experiments were carried out applying a constant voltage of 10 kV, unless stated otherwise, with anode at the inlet and cathode at the outlet side (cationic mode). Sample introduction was achieved by pressure injection with injection volumes of about 6.0 nl/s. Data analysis was performed using the laboratory-written data analysis program CAESAR.

Chemicals

All chemicals were of analytical-reagent grade. Sodium propionate was obtained from Sigma (St. Louis, MO, USA). Before making the stock solu-

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tions the sodium propionate was dried at 100°C for 30 min. Yeast, wheat flour and the all-purpose flour were obtained at a local bakery and brown sugar, margarine and salt were obtained at a local supermarket.

Bread preparation

For the preparation of a bread, 75 g of wheat flour, 50 g of all-purpose flour, 6.5 g of yeast, 2.5 g of salt, 5 g of brown sugar, 5 g of margarine and 70 g of water were mixed manually in a large bowl, until the dough was stiff enough to leave the side of the bowl. The dough was placed in a warm location for 1 h to rise, then kneaded thoroughly and was left for 30 min in the warm location to rise. The dough was then kneaded once again, shaped into the final form of the bread and allowed to rise once again for 45 min. Finally, the bread was baked in an oven at 120°C for 45 min or 225°C for 25 min. If the bread was spiked with sodium propionate, the sodium propionate was dissolved in the water, before preparing the dough.

RESULTS AND DISCUSSION

Identification of propionate

Important questions with regard to the determination of propionate in bread are whether propionate can be separated from the bread matrix and how it can be recognized. Migration times often cannot be used to identify components owing to varying velocities of the electroosmotic flow (EOF). Effective mobilities, however, can be used for peak recognition because after the calculation of the effective mobilities the effect of the EOF is eliminated. Further, effective mobilities give information on how to separate the components from the matrix if needed [4]. If no specific interactions occur, such as complex formation, the effective mobility in a specific background electrolyte can be calculated from the ionic mobility at infinite dilution and the pK value of the component. For propionate we calculated and measured the effective mobilities for several background electrolytes at different pH values. In Table I the compositions of all background electrolytes are given. For the determination of the non-UV-absorbing propionate the indirect UV mode must be applied. For this reason, all background electrolytes contain the UV-absorbing negative ionic species benzoic acid. In Fig. 1 the calculated relationship (solid line) between effective mobility and pH and several measured values of the effective mobilities are given. The ionic strength was not identical for all background electrolytes. The measured values cover the calculated values, showing the usefulness of this principle. As can be seen in Fig. 1, relatively small variations in pH, especially near the pK value of the component (the pK value of propionate is 4.87), result in large changes in effective mobilities. This explains the differences in

TABLE I

COMPOSITIONS OF BACKGROUND ELECTROLYTES AT DIFFERENT pH VALUES

All buffers were prepared by adding benzoic acid to the cations until the desired pH was reached.

Cation	pH	Cation	pH
0.005 *M* Tris	4.18	0.01 *M* Histidine	6.24
0.005 *M* Tris	4.58	0.01 *M* Histidine	6.67
0.005 *M* Tris	4.99	0.01 *M* Tris	7.05
0.005 *M* Tris	5.39	0.01 *M* Tris	7.90
0.01 *M* Histidine	5.84	0.01 *M* Tris	8.01

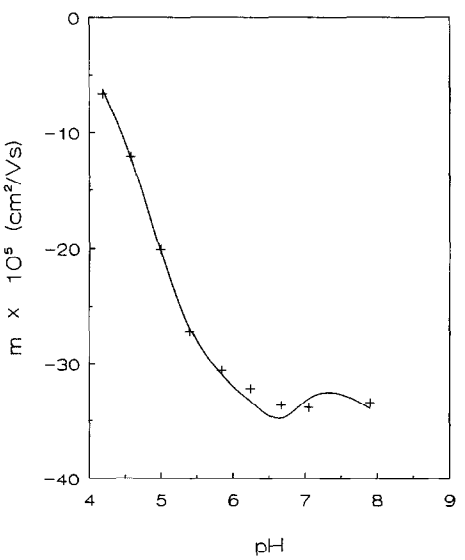

Fig. 1. Calculated relationship (solid line) between the effective mobility of propionate and pH and (+) measured values determined for several different background electrolytes.

effective mobilities obtained with different batches of background electrolytes. The effective mobility of propionate measured in the matrix was always checked by measuring the effective mobility of propionate in the standard solution for each background electrolyte.

Calibration graphs in CZE

Although for the determination of propionate in the indirect UV mode an internal standard can be applied, so that calibration graphs are superfluous [5], we studied the construction of calibration graphs. In principle a calibration graph can be set up in two ways, either by injecting equal injection volumes (or a constant pressure-injection time) of different sample concentrations or by injecting a specific sample concentration with several different injection volumes. For the construction of such a calibration graph, about six analyses have to be carried out, which is time consuming. Because CZE is an elution technique, it is possible to repeat injections during one electrophoretic run. In this way it is possible to construct a calibration graph by injecting different standard solutions (or pressure-injection times) of propionate within one electrophoretic run. Of course, this principle is only valid at a constant velocity of the EOF during the whole electrophoretic run.

To check this principle, the calibration graphs obtained in the different ways were compared. In Fig. 2 the calibration graphs of propionate are shown for (+) several different sample concentrations (in the range $5 \cdot 10^{-5}$– $5 \cdot 10^{-4}$ *M*) with a 3-s pressure-injection time, (△) injection of $5 \cdot 10^{-5}$ *M* propionate with different pressure-injection times in the range 3–30 s and (○) repeated injections (time between two injections 1 min) within one electrophoretic run of different sample concentrations with a 3-s pressure-injection time, for a background electrolyte of 0.02 *M* Tris adjusted to pH 8.0 and 0.005 *M* Tris adjusted to pH 4.6 by adding benzoic acid. The amount injected is given in m*M*s, *i.e.*, the product of concentration (m*M*) and injection time (s).

It can be seen that in both background electrolytes nearly identical calibration graphs are obtained for the three methods. In Table II the regression coefficient, slope, intercept and limit of detection [6] are given for all calibration graphs. In Fig. 3

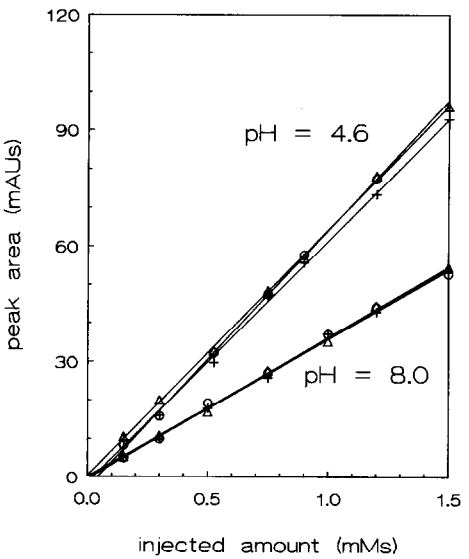

Fig. 2. Calibration graphs of propionate for (+) different sample concentrations with equal (3 s) pressure-injection time, (△) injection of $5 \cdot 10^{-5}$ *M* propionate with different pressure-injection times and (○) repeated injections (time between two injections 1 min) within one electrophoretic run of different sample concentrations with a 3-s pressure-injection time, for a background electrolyte of 0.02 *M* Tris adjusted to pH 8.0 and 0.005 *M* Tris adjusted to pH 4.6 by adding benzoic acid. The amount injected is indicated in m*M*s (molarity multiplied by pressure-injection time).

an electropherogram with a seven-times repeated injection applying a background electrolyte at pH 8.0 is given. The seven EOF dips and seven propionate dips with linearly increasing peak area can be clearly seen. It is notable that the non-UV-absorbing sodium ions in the sodium propionate solutions show a positive UV peak. According to the Kohlrausch regulation function, the total ionic concentration in a sample zone of an ion with a higher effective mobility than that of the co-ion of the background electrolyte is higher than that of the background electrolyte. According to the electroneutrality condition, the concentration of the UV-absorbing buffering counter ions is also higher, which explains the UV peaks for non-UV-absorbing sample ions. Although this background electrolyte is far from ideal for the determination of sodium ions, a regression coefficient of 0.9985 for the calibration graphs using the peak area is fairly good.

TABLE II

REGRESSION COEFFICIENT, r, SLOPE, INTERCEPT AND LIMIT OF DETECTION (LOD) FOR CALIBRATION GRAPHS OF PROPIONATE SHOWN IN FIG. 2

Background pH	Method[a]	r	Slope (AU/M)	Intercept (mAUs)	LOD (mMs)
8.0	1	0.9989	35.96	−0.284	0.0813
8.0	2	0.9992	36.71	−0.518	0.0792
8.0	3	0.9984	35.97	0.067	0.0899
4.6	1	0.9992	62.76	−1.463	0.0882
4.6	2	0.9998	63.57	0.810	0.0316
4.6	3	0.9995	66.95	−2.867	0.0848

[a] Method by which the calibration graph was obtained: 1 = using different concentrations; 2 = using one concentration and different injection times; 3 = using repeated injection method.

Matrix effects in CZE

As shown before, propionate can be recognized through its effective mobility and quantified in the indirect UV mode. In order to choose a suitable background electrolyte for the determination of propionate in bread, matrix effects have to be known. For this reason, laboratory-baked bread was prepared with and without the addition of sodium propionate before the baking process. For the extraction of the propionate from the bread matrix, in the first instance a slice of bread was taken, a specific volume of water was added, the mixture was sonicated for 10 min and analysed, after filtration, with several background electrolytes at different pH values. In fact, all background electrolytes (see Table I) can be applied for the determination of propionate in bread, although at some pH values matrix components interfere with the propionate peak. In Fig. 4, typical electropherograms of (a) a standard solution of propionate and of bread (b) without and (c) with propionate added are shown measured with background electrolytes at pH 4.6 and 8.0. It can be clearly seen that at pH 8.0 a component X of the bread matrix coincides with propionate, which is not visible (at the position of propionate) in the electropherogram measured at pH 4.6. Also, some cations (although UV peaks are visible, it may be non-UV-absorbing cations) and several anions in the indirect UV mode are present. Although owing to variation in the velocity of the EOF the migration times sometimes differ, the effective mobilities are almost constant (see also Tables III–V).

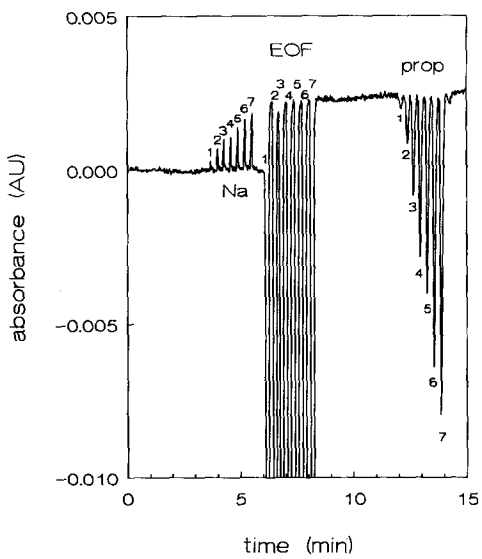

Fig. 3. Electropherogram showing the principle of repeated injections. A sample injection was made seven times (time between injections 0.3 min) with different sample concentrations with a pressure-injection time of 3 s. The seven sodium peaks (for explanation, see text), seven EOF dips and seven propionate peaks can clearly be distinguished.

Preliminary experiments showed also that the determination of propionate in a bread matrix resulted in varying velocities of the EOF, possibly owing to protein adsorption on the capillary wall. In all further quantitative experiments we therefore applied calibration graphs measured with identical pressure-injection times of 3 s and different concentrations, whereby temporal peak areas were recalculated to spatial peak areas [7]. Before all experiments rinsing steps for 2 min with 1 M KOH, for 2

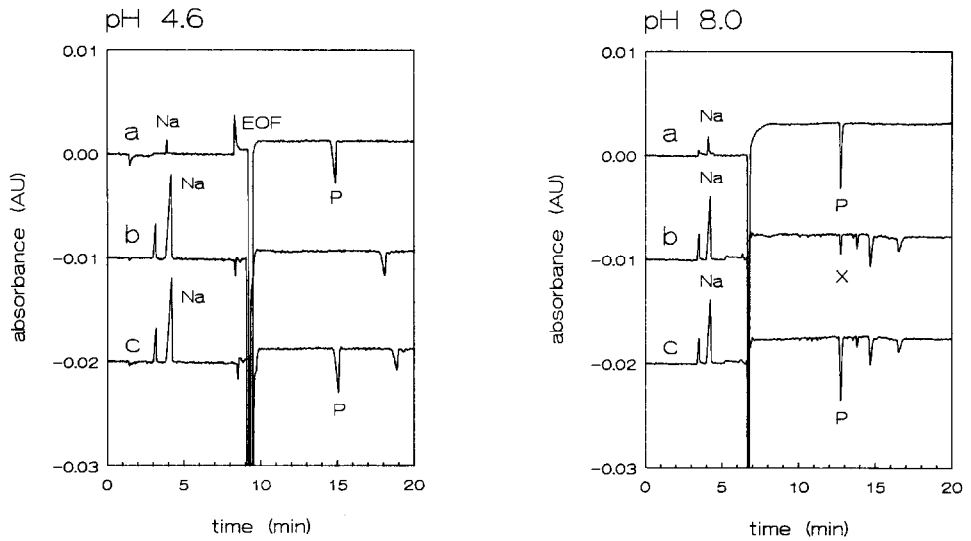

Fig. 4. Typical electropherograms of (a) a standard solution of propionate and of bread (b) without and (c) with propionate added, measured with background electrolytes at pH 4.6 and 8.0. P = propionate; Na = sodium; X = unknown component.

min with water and for 3 min with background electrolyte were carried out.

Extraction procedure and recovery

To extract the propionate from the bread, a slice of bread (containing a proportional part of crust and inside) was weighed accurately and a volume of water that was ten times the mass of the bread was added. After sonication and filtration, the sample solution was diluted in order to obtain a concentration near the centroid of the calibration graphs. The determined values for propionate were recalculated to milligrams of propionate per gram of bread (the mass of a bread was measured after the baking process). In order to study the effect of extraction time, recoveries were measured after several sonication times. In Table III the measured values applying a background electrolyte at pH 4.6 are given, showing an average recovery of about 80% with a sonication time of more than 20 min.

TABLE III

DETERMINED AMOUNT, RECOVERY OF THE EXTRACTION OF SODIUM PROPIONATE IN BREAD (ADDED AMOUNT 2.211 mg/g BREAD) AND CALCULATED EFFECTIVE MOBILITY AS A FUNCTION OF TIME OF SONICATION

Applied background electrolyte, 0.005 *M* Tris–benzoate (pH 4.6).

Time (min)	Determined (mg/g bread)	Recovery (%)	m_{eff} (10^{-5} cm^2/V · s)
10	1.634	73.9	−12.41
20	1.763	79.7	−12.38
30	1.779	80.5	−12.37
40	1.805	81.6	−11.99
50	1.809	81.8	−12.33

To study whether the recovery of *ca.* 80% is connected with a distribution equilibrium of propionate over bread and water phase or whether part of the propionate disappeared during the baking process, recoveries were measured for bread baked at 120°C and 225°C applying both a single extraction step and a five-times repeated extraction step. In the single extraction step 100 g of water were added to 10 g of bread, followed by sonication for 30 min and filtration. In the repeated extraction step, 100 g of water were added to 10 g of bread, followed by sonication for 10 min. After filtration of the bread this step was repeated four times and all fractions of filtered water were collected and analysed.

All the results are given in Table IV. From the

TABLE IV

ADDED AMOUNT OF SODIUM PROPIONATE, DETERMINED AMOUNT OF SODIUM PROPIONATE, RECOVERY FOR THE FIVE BREADS, BAKED AT 225 AND 120°C, AND EFFECTIVE MOBILITY

Applied background electrolyte, 0.005 *M* Tris–benzoate (pH 4.6).

Baking temperature (°C)	Bread No.	Added (mg/g bread)	Single extraction			Repeated extraction		
			Determined (mg/g bread)	Recovery (%)	m_{eff} (10^{-5} cm^2/V · s)	Determined (mg/g bread)	Recovery (%)	m_{eff} (10^{-5} cm^2/V · s)
225	1	0	0	–	–	0	–	–
	2	0.744	0.604	81.3	−12.42	0.702	94.4	−12.26
	3	1.452	1.180	81.2	−12.42	1.427	98.3	−12.69
	4	2.211	1.772	80.2	−12.27	2.017	95.6	−12.21
	5	6.947	5.847	84.2	−12.27	6.589	94.8	−12.21
120	1	0	0	–	–	0	–	–
	2	0.721	0.619	85.8	−12.45	0.709	98.3	−12.16
	3	1.413	1.249	88.4	−12.51	1.413	94.3	−12.23
	4	2.122	1.847	87.0	−12.40	2.122	98.5	−12.12
	5	6.837	6.723	83.6	−12.45	6.190	90.5	−12.79

recoveries of *ca.* 95% for the repeated extraction for the breads baked at both 120 and 225°C it can be concluded that during the baking process no loss of propionate occurs and recoveries of *ca.* 80% for a single extraction or *ca.* 95% for repeated extraction could be established.

Determination of propionate in commercially available bread

To test the applicability of the method we determined propionate in five breads from a local bakery and four kinds of pumpernickel, applying the five-times repeated extraction procedure using the background electrolyte at pH 4.6 in order to avoid interfering components possibly present in the bread matrix. The results are given in Table V. As can be seen, propionate was found only in the pumpernickel and not in the breads from the local bakery.

CONCLUSIONS

CZE is a suitable separation technique for the determination of propionate in bread. With a repeated extraction procedure propionate can be ex-

TABLE V

DETERMINED AMOUNT OF SODIUM PROPIONATE AND EFFECTIVE MOBILITY FOR COMMERCIAL BREADS

Applied background electrolyte, 0.005 *M* Tris–benzoate (pH 4.6).

Bread	Determined amount (mg/g bread)	m_{eff} (10^{-5} cm^2/V · s)
White bread 1	–	–
White bread 2	–	–
Whole-meal bread	–	–
White bread roll	–	–
Whole-meal bread roll	–	–
Pumpernickel 1	1.71	−12.03
Pumpernickel 2	0.72	−12.14
Pumpernickel 3	1.69	−12.05
Rheinisches Schwarzbrot	0.64	−12.06

tracted from the bread matrix with an average recovery of *ca.* 95%. For peak recognition the effective mobility can be used. The procedure has been tested with laboratory-baked bread containing known amounts of propionate.

One of the problems encountered in the propionate determination is the possible presence of interfering components in the bread matrix. At pH 4.6 no matrix components interfering with propionate were measured in the laboratory-baked bread. To show the applicability of the method, propionate was determined in five different commercial breads from the local bakery and four different kinds of pumpernickel. Only in the prepacked pumpernickels propionate was found, with amounts below the allowed limit of 0.3%.

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