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RAPID COMMUNICATION

The application of capillary electrophoresis to the determination of total niacin in concentrated yeast spreads

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A rapid capillary electrophoretic method for the determination of total niacin in concentrated yeast spreads has been developed. Nicotinic acid is liberated from the food matrix by autoclaving the sample in aqueous calcium hydroxide. The sample solution is then passed through an activated C18 Sep-Pak cartridge followed by cation exchange chromatography using commercially available SCX columns. The nicotinic acid then is determined by capillary electrophoresis using a 75 cm×75 μm uncoated fused silica capillary column with a buffer comprising of a 1:1 mixture of 0.02 M sodium tetraborate and 0.02 M disodium hydrogen orthophosphate pH 9.2. The levels of nicotinic acid determined by capillary electrophoresis were in good agreement with those obtained by the traditional AOAC colorimetric procedure (e.g. CE 126 mg/100 g, AOAC 129 mg/100 g).

INTRODUCTION

Niacin occurs naturally in a wide variety of foods, either bound chemically or as free nicotinic acid or as nicotinamide (Ball, 1994). To determine the total niacin content of a particular food, the bound form of the vitamin and any nicotinamide present in the food must be converted to nicotinic acid, by treatment with either acid or alkali. The liberated nicotinic acid can then be determined, either by the AOAC colorimetric procedure using cyanogen bromide and sulphanilic acid (Williams, 1984), or by the microbiological assay using *Lactobacillus plantarum* (Ball, 1994). A number of methods utilizing high-performance liquid chromatography (HPLC) with ultraviolet (UV) absorption as the determining step also have been used to determine the niacin content in selected foods. The sample matrix, clean-up, HPLC procedures and references are given in Table 1. Gas chromatography also has been used to determine the level of added nicotinamide in meat products. The nicotinamide was extracted with acetonitrile before conversion to 3-cyanopyridine with heptafluorobutyric anhydride (Tanaka *et al.*, 1989). A recent publication by Ball (1994) gives an excellent description of the chemical and biological nature of niacin, as well as aspects of the analysis of the vitamin in a range of commodities.

Recently, methods based on the capillary electrophoretic (CE) method as the determining procedure have been used to quantify a wide variety of additives and naturally occurring compounds present in foods (Lindeberg, 1996*a*,*b*). The CE methods are often faster and more specific than the corresponding HPLC procedures (Thompson & Trenerry, 1995*a*,*b*; Marshall *et al.*, 1995; Thompson *et al.*, 1995). Micellar electrokinetic capillary chromatography (MECC) was used to determine nicotinic acid in a vitamin preparation using an uncoated fused silica column with a 0.02 M phosphate buffer pH 9.0 containing 0.05 M sodium dodecyl sulphate (SDS) (Fujiwara *et al.*, 1988) and in human plasma with either a 0.01 M sodium tetraborate buffer pH 9.36 or 10% acetonitrile/90% 0.01 M potassium dihydrogen orthophosphate buffer pH 2.5 (Zarzycki *et al.*, 1995). The migration behaviour of nicotinic acid and some of its derivatives was investigated using CE by Tanaka and co-workers (Tanaka *et al.*, 1995). Nicotinic acid also has been separated from other water-soluble vitamins by CE (Jegle, 1993) and by microemulsion electrokinetic chromatography (Boso *et al.*, 1995).

This paper describes the determination of total niacin in concentrated yeast spreads by CE. These products are an excellent source of B group vitamins and are used as spreads on toast and bread and can be added to soups, stews and gravies as flavour enhancers. The nicotinic acid is liberated from the food by alkaline digestion and

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separated from interfering compounds by solid phase extraction using commercially available C18 cartridges and cation exchange (SCX) columns. A similar method based on a MECC separation also is described. The levels determined by CE are compared with those determined by the standard AOAC colorimetric procedure (Williams, 1984).

MATERIALS AND METHODS

Reagents

The SDS, sodium deoxycholate, nicotinic acid and sodium saccharin were obtained from Sigma Chemical Co., St Louis, MO, USA. Disodium hydrogen orthophosphate was obtained from BDH Chemicals, Kilsyth, Australia. Oxalic acid, sodium tetraborate and potassium dihydrogen orthophosphate were obtained from Ajax Chemicals, Auburn, Australia. All chemicals and solvents were AR grade or HPLC grade and were used without further purification. The C18 Sep-Pak Vac cartridges (500 mg) were obtained from Waters Corporation, Milford, MA, USA. The SCX cation exchange columns (500 mg) were obtained from Varian, CA 90710, USA.

Preparation of standards, samples and buffers

Standards

A 100 μg/ml stock standard solution of nicotinic acid was prepared by dissolving 20 mg of dry nicotinic acid in 25% ethanol in deionized water and made to a final volume of 200 ml. The solution was stored in the refrigerator. Working standards of between 1 and 15 μg/ml were prepared by diluting the stock solution with deionized water. Suitable aliquots of a 400 μg/ml stock solution of saccharin (internal standard) were added during the preparation of the working standards to produce a final concentration of 40 μg/ml for the internal standard.

Samples and sample preparation

The samples were purchased from local outlets and analysed before the 'used-by' dates.

MECC

To approximately 1 g of the yeast spread was added 0.75 g of calcium hydroxide and 20 ml of deionized water. For recovery tests, 5 ml of the 100 μg/ml standard nicotinic acid solution was added. The mixture was mixed thoroughly and autoclaved for 2 h at 121°C (~104 kPa) and mixed while still hot. The cooled mixture was diluted to 50 ml with deionized water, thoroughly mixed, and centrifuged at 2500 rpm for 15 min at 0°C. A 20 ml aliquot of the supernatant was adjusted to pH 7 by adding saturated aqueous oxalic acid (~10%) and made to 25 ml with deionized water. The resultant suspension was centrifuged at 2000 rpm for 10 min at 0°C and a 5 ml sample of the supernatant was diluted to 50 ml with water. A 10 ml portion of this solution was passed through a SCX cation exchange column (500 mg) which had been conditioned previously with 10 ml methanol followed by 10 ml deionized water. The column was washed with 5 ml of deionized water followed by 5 ml of methanol. Nicotinic acid was eluted from the SCX column by washing the column with 5 ml of freshly pre- pared 2% concentrated ammonium hydroxide in methanol. The solvent was removed under a stream of nitrogen and the residue dissolved in 1 ml of a 40 μg/ml aqueous saccharin solution. The solution was filtered through a 0.8 μm cellulose acetate filter disc before analysis.

CE

The samples were treated as described for the MECC determination, except that a 10 ml portion of the solution was passed through a C18 Sep-Pak Vac Cartridge (500 mg) and a SCX column (500 mg) connected in series. The columns were conditioned with 10 ml methanol followed by 10 ml deionized water. The columns were washed with 5 ml of water, the C18 Sep-Pak cartridge discarded, and the SCX column washed with 5 ml of methanol. Nicotinic acid was eluted from the SCX column as described for the MECC determination.

Colorimetric procedure

The niacin content of the samples was determined as described by Williams (1984).

Table 1. Summary of the various clean-up and HPLC procedures used for the determination of niacin in food and other matrices

Matrix	Clean-up	HPLC	Reference
Cereals	Anion exchange	Reverse phase ion pair	Tyler & Shrago, 1980
Instant coffee	C18 Sep-Pak cartridge	Reverse phase ion pair	Trugo *et al.*, 1985
Meat	Nil	Reverse phase ion pair	Takatsuki *et al.*, 1987
Meat	Nil	Strong cation exchange	Hamano *et al.*, 1988
Fortified foods	Nil	Reverse phase ion pair	Rees, 1989
Beef, semolina, cottage cheese	C18 Sep-Pak cartridge	Reverse phase ion pair	Tyler & Genzale, 1990
Legumes, pork muscle	Anion exchange	Reverse phase ion pair	Vidal-Valverde & Reche, 1991
Compound feed	Cation exchange	Ion interaction	Fontaine & Horr, 1993
Urine	Cation exchange	Reverse phase ion pair	Iwaki *et al.*, 1994
Human plasma	Nil	Reverse phase ion pair	Zarzycki *et al.*, 1995

Buffer for MECC
A 2.16 g sample of sodium deoxycholate was dissolved in 100 ml of a 1:1 mixture of 0.02 M sodium tetraborate and 0.02 M potassium dihydrogen orthophosphate. The pH of the solution was 8.6. The solution was filtered through a 0.8 μm cellulose acetate filter disc before use.

Apparatus

MECC
The extracts were analysed using a 75 cm×75 μm uncoated fused silica capillary column with an effective length of 50 cm to the detector (Polymicro Technologies, Arizona, USA), fitted to an Isco Model 3140 Electropherograph (Isco Inc., Lincoln, NE, USA). The separations were performed at 20 kV and at 30°C. The compounds were loaded under vacuum (vac. level 2, 25 kPa/s) and were detected at 254 nm and at 0.01 AUFS. The capillary was flushed with running buffer for 2 min between runs. Electropherograms were recorded with the ICE Data Management and Control software supplied with the Model 3140 Electropherograph. Peak areas were used in the calculations.

A Hewlett–Packard 3D Capillary Electropherograph equipped with a photodiode array detector and fitted with a 75 μm fused silica column with an effective length of 66 cm to the detector was used to obtain the UV spectra and peak purity data.

CE
The extracts were analysed with the same conditions as described for MECC, except that the buffer was a 1:1 mixture of 0.02 M sodium tetraborate and 0.02 M disodium hydrogen orthophosphate, pH 9.2.

RESULTS AND DISCUSSION

MECC

Our initial attempts to determine the total nicotinic acid content of concentrated yeast spreads by MECC was based on a separation described by Fujiwara *et al.* (1988). The authors were able to separate nicotinic acid and nicotinamide from a number of other water-soluble vitamins in standard solutions and in a commercial vitamin preparation with an uncoated fused silica capillary using a buffer consisting of 0.02 M phosphate solution pH 9.0 containing 0.05 M SDS as the micelle modifier. The vitamins were detected by UV at 254 nm.

The sample of yeast spread first was subjected to autoclaving in the presence of aqueous calcium hydroxide in order to release any bound forms of nicotinic acid and to hydrolyse any added nicotinamide. The pH of the solution was adjusted to 7 with saturated oxalic acid (to remove the dissolved calcium salts) and the solution diluted with deionized water and analysed. Saccharin was used as the internal standard to assist in peak identification and quantification. The samples also were analysed by the traditional colorimetric technique (Williams, 1984) and by an HPLC method based on that described by Tyler & Genzale (1990). The results were encouraging (e.g. MECC 113 mg/100 g, colorimetric 105 mg/100 g, HPLC 142 mg/100 g). Figure 1 shows the separation of nicotinic acid and saccharin in the sample extract using the 0.05 M SDS/0.02 M disodium orthophosphate buffer pH 8.8 as well as the UV spectrum of the nicotinic acid in the extract compared with the library UV spectrum of pure nicotinic acid. The spectral match was quite poor (813 out of a possible 1000), indicating the presence of an extra compound either partially of fully co-migrating with nicotinic acid. We were unable to repeat the determination consistently, with the most probable cause of error being the presence of the extra peak affecting the integration. Passing the extract through an activated C18 Sep-Pak cartridge failed to remove the extra peak from the electropherogram. Changing the buffer to a 1:1 mixture of 0.02 M potassium dihydrogen orthophosphate and 0.02 M sodium tetraborate pH 8.6 and replacing the SDS with sodium deoxycholate as the micelle modifier also had little affect on the separation. We then looked at modifying both the MECC conditions and the clean-up procedure in an attempt to increase the repeatability of the determination.

Addition of organic solvent modifier to the buffer
The addition of organic solvents to MECC buffers has been shown to dramatically affect the separation of the components of complex mixtures (Thompson & Trenerry, 1995*b*; Trenerry *et al.*, 1995). The addition of 10% acetonitrile to the phosphate/borate buffer containing sodium deoxycholate induced baseline separation of the nicotinic acid from the previously partially co-migrating compound (Fig. 2). The purity of the peak corresponding to nicotinic acid was confirmed by spectral analysis of the peak. For this separation, the UV spectrum of the nicotinic acid in the sample extract was a much better match (996 out of a possible 1000) with the library spectrum of nicotinic acid. Saccharin had a slightly longer migration time than nicotinic acid and was shown to be a suitable internal standard for the quantification. The levels of nicotinic acid in three different products of concentrated yeast spread were in good agreement with those determined with the colorimetric procedure (product 1, MECC 117 mg/100 g, AOAC 121 mg/100 g; product 2, MECC 105 mg/100 g, AOAC 123 mg/100 g; product 3, 108 mg/100 g, AOAC 104 mg/100 g). The MECC separation remained consistent over twenty repetitive injections. In contrast to the acetonitrile, the addition of 10% dimethylformamide to the buffer had no affect on the separation of the two partially co-migrating peaks.

Sample clean-up using cation exchange chromatography
Cation exchange chromatography was used as a clean-up procedure for the determination of nicotinic acid in

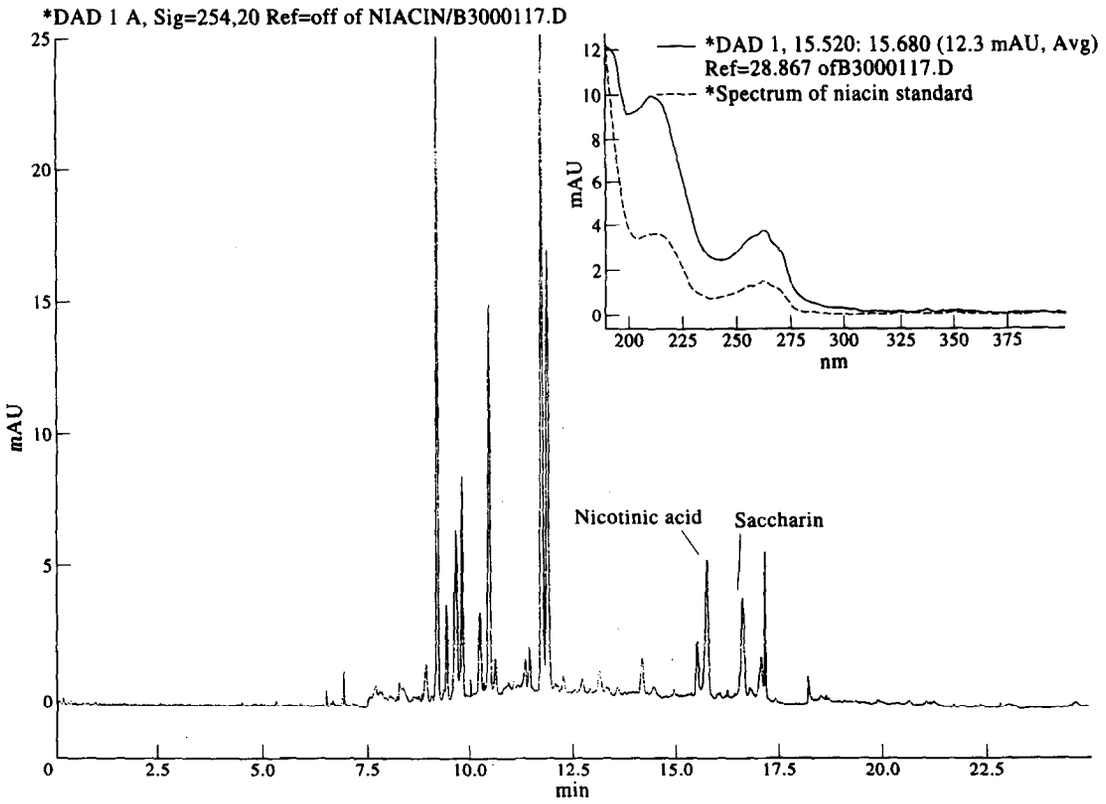

Fig. 1. Electropherogram showing the separation of nicotinic acid and saccharin (internal standard) by MECC and a comparison of the UV spectra of the peak corresponding to nicotinic acid and pure nicotinic acid.

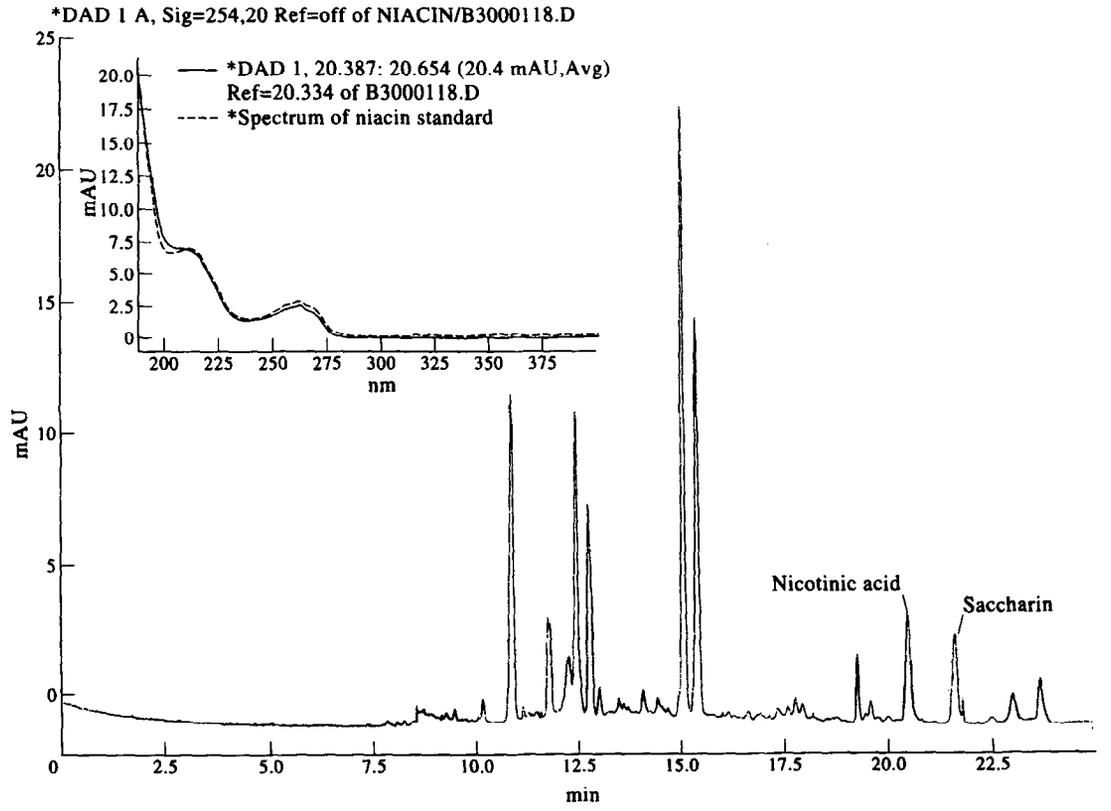

Fig. 2. Electropherogram showing the separation of nicotinic acid and saccharin (internal standard) by MECC using a buffer modified with 10% acetonitrile and a comparison of the UV spectra of the peak corresponding to nicotinic acid and pure nicotinic acid.

urine by HPLC (Iwaki *et al.*, 1994). The nicotinic acid was trapped on an activated SCX column, washed with water followed by 40% methanol in water and finally eluted with 2% concentrated ammonium hydroxide in methanol.

This technique was applied to the concentrated yeast spread extract after the removal of the excess calcium by the addition of saturated oxalic acid. After loading an aliquot of the extract onto the column, the column was washed with water and then methanol to remove unwanted compounds. Then the nicotinic acid was removed from the column with 2% concentrated ammonium hydroxide in methanol. The solvent was removed with a stream of nitrogen and the residue dissolved in deionized water containing an appropriate amount of internal standard (saccharin). The solution was filtered and analysed by MECC using the deoxycholate/phosphate/borate buffer. The resulting electropherogram was much cleaner than the electropherogram recorded before the ion exchange clean-up and showed only one peak which corresponded to nicotinic acid (Fig. 3). The peak was shown to be pure by spectral analysis using the photodiode array detector (library match 996 out of a possible 1000). The levels of nicotinic acid in three different samples determined with this work-up procedure also were in reasonable agreement with those determined by the AOAC colorimetric procedure (product 1, MECC 134 mg/100 g, AOAC 121 mg/100 g; product 2, 129 mg/100 g, AOAC 123 mg/100 g; product 3, 108 mg/100 g, AOAC 104 mg/100 g). Recoveries of nicotinic acid that were added to the samples prior to autoclaving with aqueous calcium hydroxide were: product 1, 115%; product 2, 96% and product 3, 103%).

CE

Capillary electrophoresis also has been used to separate nicotinic acid from other water-soluble vitamins (Jegle, 1993). Separation of compounds by capillary electrophoresis is determined primarily by the electrophoretic behaviour of the analytes, whereas MECC separations are also influenced by interactions between the analytes and the micelle modifier. We decided to investigate the potential of CE for the determination of total nicotinic acid in concentrated yeast spreads, as it was discovered that the use of a micelle did not significantly affect the overall separation of peaks in the electropherograms.

Figure 4 shows the separation of nicotinic acid in the initial digest (adjusted to pH 7 with saturated aqueous oxalic acid) of a sample of concentrated yeast spread using a buffer consisting of a 1:1 mixture of 0.02 M disodium hydrogen orthophosphate and 0.02 M sodium tetraborate buffer pH 9.2. The UV spectrum of nicotinic acid was an excellent match with the library spectrum of nicotinic acid (Fig. 4). However, the remainder of the

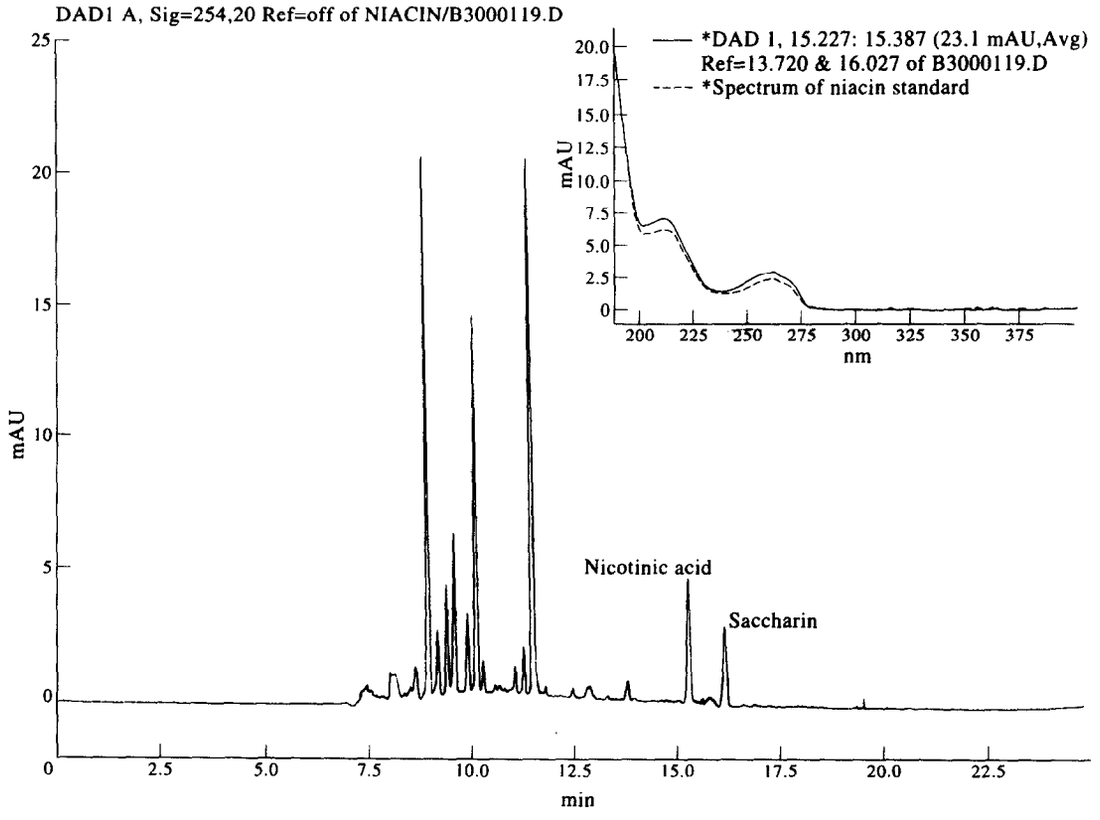

Fig. 3. Electropherogram showing the separation of nicotinic acid and saccharin (internal standard) by MECC using a buffer described in the Materials and methods and a comparison of the UV spectra of the peak corresponding to nicotinic acid after SCX column clean-up and pure nicotinic acid.

electropherogram was quite complex, especially in the region where the internal standard (saccharin) was expected to migrate. Passing the digest (pH 7) through an activated C18 Sep-Pak Vac cartridge followed by ion exchange chromatography with a SCX cation exchange column produced a much cleaner electropherogram and also permitted the use of saccharin as the internal standard (Fig. 5). The purified yeast spread extracts were analysed by CE using a variety of buffers ranging in pH from 6.8 to 9.3. The change in pH of the buffers did not greatly affect the migration times or the peak shapes of nicotinic acid and saccharin. The final analyses were carried out using a buffer consisting of a 1:1 mixture of 0.02 M disodium hydrogen orthophosphate and 0.02 M sodium tetraborate pH 9.2 because it provided the best overall separation of peaks in the electropherograms. The CE separation was very reproducible. The migration times and the separation of nicotinic acid and saccharin in the sample extract were consistent and were maintained over 20 repetitive injections. The CE column was flushed with running buffer between runs to remove unwanted compounds that migrated after saccharin. To test the suitability of the CE conditions for quantitative purposes, a number of standard solutions of different concentrations containing saccharin (40 μg/ml) as the internal standard were analysed seven times. The detector response was linear from 0.5 μg/ml to at least 15 μg/ml nicotinic acid. The C.V. data for peak area calculation were also acceptable for nicotinic acid in the concentration range 1–15 μg/ml (1 μg/ml, C.V. 3.1%; 2 μg/ml, C.V. 4.3%; 5 μg/ml, C.V. 2.6%; 10 μg/ml, C.V. 0.8%; 15 μg/ml, C.V. 1.5%).

The procedure then was used to quantify the level of nicotinic acid in three different concentrated yeast spreads. The electropherograms obtained showed one peak corresponding to nicotinic acid and this peak was shown to be pure by spectral analysis using the photodiode array detector. The values compared favourably with those determined by the standard AOAC colorimetric procedure (product 1, 126 mg/100 g, AOAC 129 mg/100 g; product 2, 114 mg/100 g, AOAC 123 mg/100 g; product 3, 100 mg/100 g, AOAC 104 mg/100 g). The recovery of nicotinic acid added to product 1 prior to autoclaving with aqueous calcium hydroxide was 96%. Seven portions of product 1 were analysed in the one batch to check the repeatability of the method. The resultant C.V. of 3.3% indicated that the method was robust and repeatable.

We chose to develop and use the solid phase extraction clean-up/CE procedure for the determination of total niacin in these products in preference to MECC as it was simpler, more robust and offered greater scope for the isolation, concentration and determination of nicotinic acid at naturally occurring levels (0.5–20 mg/100 g) in other foods. We are actively working on this approach for determining the levels of niacin in foods that are good sources of the vitamin, e.g. meat, cereals and cereal products, fish and nuts.

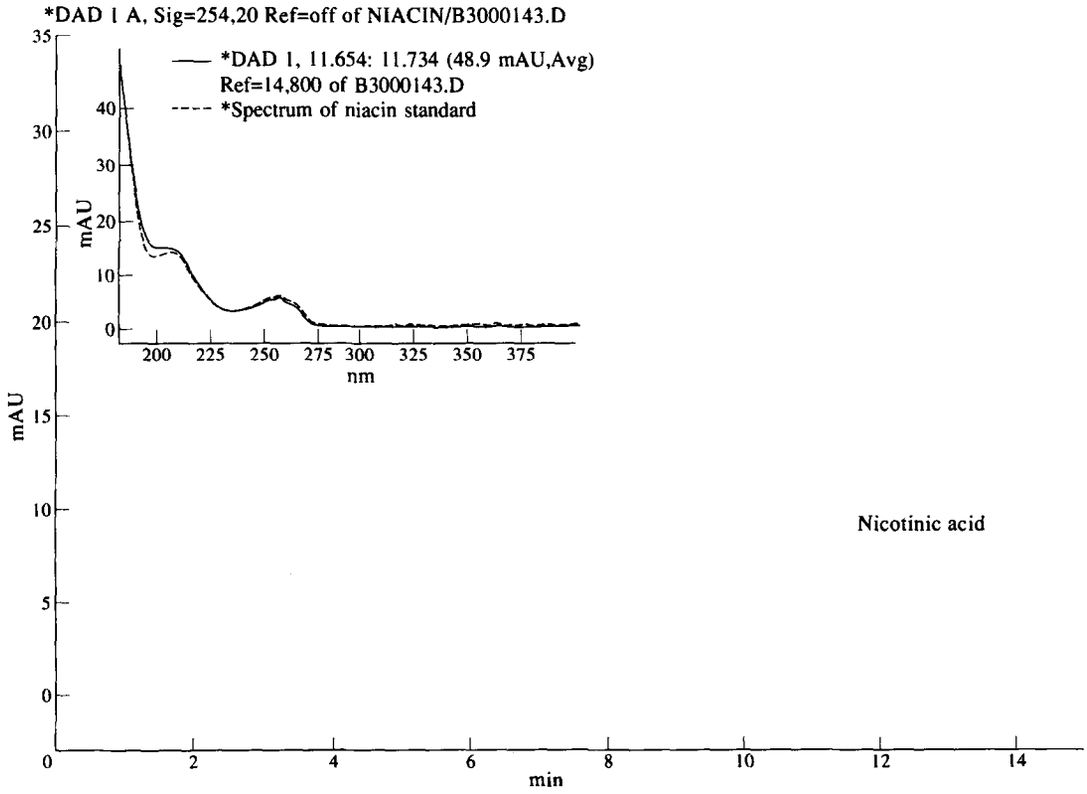

Fig. 4. Electropherogram showing the separation of nicotinic acid by CE using a buffer described in the Materials and methods section and a comparison of the UV spectra of the peak corresponding to nicotinic acid and pure nicotinic acid.

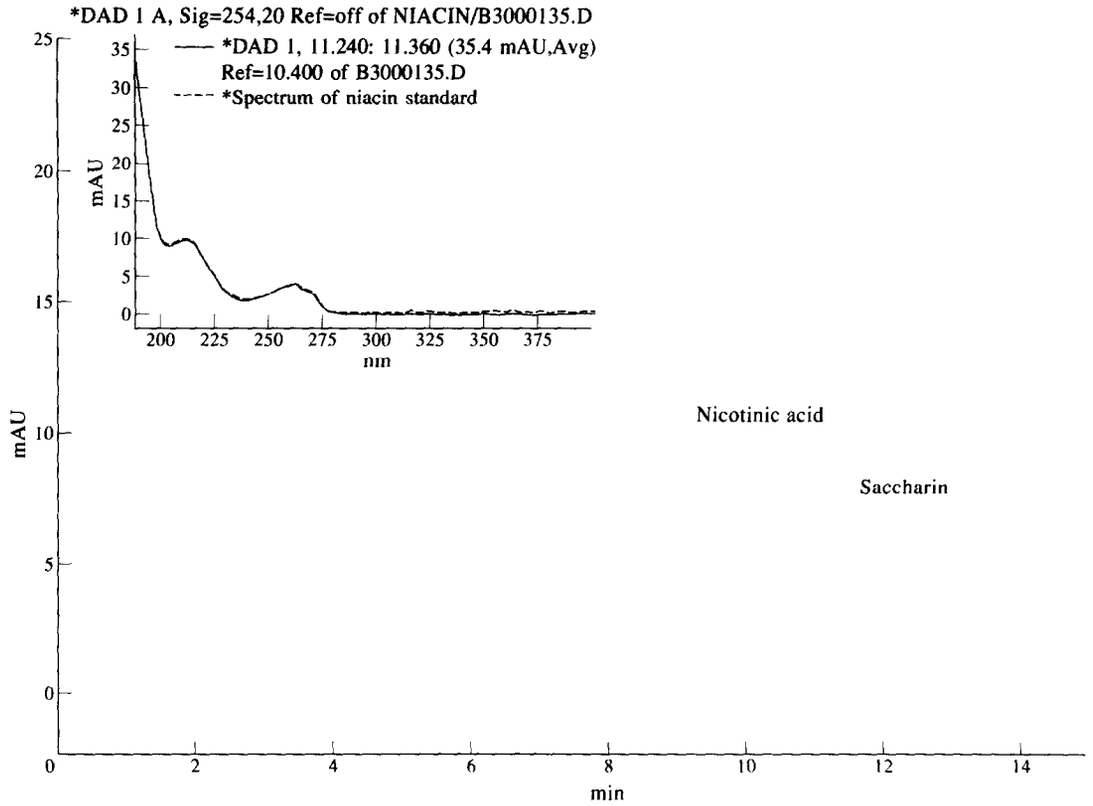

Fig. 5. Electropherogram showing the separation of nicotinic acid and saccharin (internal standard) by CE using a buffer described in the Materials and methods section and a comparison of the UV spectra of the peak corresponding to nicotinic acid after C18 Sep-Pak and SCX column clean-up and pure nicotinic acid.

CONCLUSION

A rapid capillary electrophoretic method for the determination of total niacin in concentrated yeast spreads has been developed. Nicotinic acid is liberated from the food matrix by autoclaving the sample in aqueous calcium hydroxide. Solid phase extraction using commercially available C18 and SCX cartridges was used to purify the extract prior to analysis by CE. The CE determinative step was more robust and used in preference to a similar MECC determination. The levels of niacin in the concentrated yeast spreads were in good agreement with the values obtained from the standard AOAC colorimetric procedure.

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