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The determination of niacin in selected foods by capillary electrophoresis and high performance liquid chromatography: acid extraction

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Abstract

A robust method for the determination of niacin in raw and cooked meat and fish samples using acid extraction to liberate the niacin from the food matrix and capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) as the determinative steps is described. Niacin is liberated from the food matrix by autoclaving in the presence of 0.8 M sulphuric acid at 121°C for 2 h. C18 and cation exchange solid phase extraction (SPE) cartridges were used to isolate and concentrate the niacin before analysis. CE analyses were performed with an uncoated 50 μm extended light path fused silica capillary column and a buffer comprising of 7.5% acetonitrile and 92.5% of a 1:1 mixture of 0.02 M potassium dihydrogen orthophosphate and 0.02 M disodium hydrogen orthophosphate pH 7. Saccharin was used as the internal standard. The levels of niacin determined by CE compared favourably with those determined by HPLC (canned ham CE 3.5 mg/100 g, HPLC 3.3 mg/100 g; raw lamb CE 7.7 mg/100 g, HPLC 7.7 mg/100 g). The average recovery of niacin added to the samples prior to extraction was 103%. The limit of reporting for the determination is 0.5 mg/100 g. The procedure was suitable for only a limited number of other food types (e.g. fruit, vegetables and nuts). Alkali extraction of these foods using saturated calcium hydroxide followed by SPE cleanup and CE determination provided more reliable results. The limit of reporting for this procedure was 0.2 mg/100 g for fruit and 0.5 mg/100 g for vegetables.

1. Introduction

We recently reported the determination of niacin in cereals, meat and selected foods by autoclaving the food with saturated calcium hydroxide solution at 121°C for 2 h followed by solid phase extraction (SPE) and using either capillary electrophoresis (CE) or high performance liquid chromatography (HPLC) as the determinative step (Ward & Trenerry, 1997). Under these conditions, any nicotinamide present in the sample, as well as the bound niacin, was converted to nicotinic acid. Solid phase extraction (SPE) with the C18 and SCX cartridges produced a suitable extract for CE and HPLC analysis. Nicotinic acid was well separated from interfering compounds in the resulting electropherograms and chromatograms. CE was the preferred technique as the peak shapes were better and the run times much shorter.

The alkali extraction procedure determined the total amount of niacin in the food. It is generally considered that only the acid-hydrolysable forms of niacin are fully bioavailable for humans; thus if the nutritive value is to be determined, an acid hydrolysis is preferred. Also, tryptophan can be converted to niacin in the body, and therefore the total niacin activity of a food can be determined only if the contribution of tryptophan is taken into account (Greenfield & Southgate, 1992).

This paper describes a method for the determination of niacin in raw and cooked meat and fish and a limited range of other foods using an acid extraction followed by SPE clean-up and determination by CE. The samples were also analysed by HPLC using a procedure based on that described by Tyler and Shrago (1980). Previously unpublished data on the levels of niacin in fruits

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and vegetables determined by CE after alkali extraction are also reported.

2. Materials and methods

2.1. Reagents

Nicotinic acid and sodium saccharin were obtained from Sigma Chemical Co., St Louis, MO, USA. Disodium hydrogen orthophosphate and barium hydroxide were obtained from BDH Chemicals, Kilsyth, Australia. Potassium dihydrogen orthophosphate was obtained from Ajax Chemicals, Auburn, Australia. PIC A Reagent and C18 Sep-Pak Vac cartridges (500 mg) were supplied by Waters Corporation, Milford, MA, USA. C18 Mega Bond Elut cartridges (1 g) and SCX cation exchange columns (1 g and 500 mg) were obtained from Varian, CA, USA. All other chemicals and solvents were AR grade or HPLC grade and were used without further purification. AACC Standard Reference Sample VMA 195 was obtained from the American Association of Cereal Chemists, St. Paul, MN, USA. The mean level of niacin in this sample was 18.0 mg/100 g with a range between 13.9-20.1 mg/100 g ($n = 13$). The sample was divided into aliquots of approximately 1 g and stored at -18°C until required.

2.2. Preparation of standards, samples and buffer

2.2.1. Standards

A 100 μg/ml stock standard solution of nicotinic acid was prepared by dissolving 20 mg of dry nicotinic acid in 200 ml of deionised water. The solution was stored in the refrigerator at 4°C. Working standards of between 1 and 50 μg/ml were prepared by diluting the stock solution with deionised water. Saccharin was added as the internal standard at a final concentration of 40 μg/ml for CE analysis. No internal standard was used for the HPLC determinations. The detector response for nicotinic acid was linear to at least 50 μg/ml for CE and at least 100 μg/ml for HPLC.

2.3. Samples and sample preparation

The samples were purchased from local outlets and analysed within the recommended "use by" dates. Meat and fish samples (except for canned ham) were divided into two approximately equal portions, one for analysis as the raw product and the other for analysis after cooking. The meat and fish samples were fried (no added oil) for approximately 40 min. The vegetables were cooked in a covered container in a 650 W microwave oven on "high" for 5–6 min. The broccoli, potato and pumpkin were cut into "standard serve" pieces and the corn cooked whole. A small amount of water was added to the peas and beans before cooking. Excess water was drained from the cooked food before the samples were homogenised. All samples were homogenised in a commercial food processor. The samples were stored at 4°C and analysed as soon as possible after preparation.

2.3.1. Acid extraction

To approximately 1 g of food (3 g of fruit) 10 ml of 2 M sulfuric acid was added and the volume adjusted to 25 ml with deionised water. For recovery tests, 1 ml of a 100 μg/ml nicotinic acid solution was added. The mixture was thoroughly mixed and heated in an autoclave for 2 h at 121°C (~104 kPa). The cooled mixture was diluted to 50 ml with deionised water, mixed thoroughly and centrifuged at 2500 rpm for 15 min at 0°C. A 15 ml aliquot of the supernatant was adjusted to pH 7 with saturated barium hydroxide solution (final adjustment with dilute solution) and made to 100 ml with deionised water. The resultant suspension was centrifuged at 2500 rpm for 10 min at 0°C. A C18 Sep-Pak Vac Cartridge (500 mg) was placed on top of a SCX column (500 mg) and the columns were conditioned with 10 ml methanol followed by 10 ml deionised water. For the vegetable and fruit samples, a Mega Bond Elut C18 cartridge (1 g) and an SCX column (1 g) were employed. With the exception of the vegetable samples (in which 40 ml was loaded), a 20 ml aliquot of the supernatant was loaded onto the C18 column at a flow rate of 1–2 drops per s. Water was passed through the columns at a flow rate of 1–2 drops per s, the C18 Sep-Pak cartridge discarded, and the SCX column washed with 5 ml of methanol at a flow rate of 1–2 drops per s. Nicotinic acid was removed from the SCX column with 5 ml of freshly prepared 2% solution of concentrated ammonium hydroxide in methanol. The solvent was evaporated to dryness under a stream of nitrogen at room temperature. The residue was dissolved in 1 ml of a 40 μg/ml aqueous saccharin solution for CE analysis or 1 ml of deionised water for HPLC analysis. The solutions were filtered through 0.8 μm cellulose acetate filter discs before analysis.

2.3.2. Alkali extraction

The samples were analysed by the procedure of Ward and Trenerry (1997), except that 3 g of the fruit samples were extracted and 1 g SPE cartridges were used in the clean-up step.

2.4. Buffer

The CE buffer was prepared by mixing 3.75 ml of acetonitrile and 46.25 ml of a 1:1 mixture of 0.02 M potassium dihydrogen orthophosphate and 0.02 M disodium hydrogen orthophosphate. The buffer was filtered through a 0.45 μm teflon filter disc before use.

2.5. Apparatus

2.5.1. CE

Electrophoretic experiments were performed on a Hewlett–Packard 3D capillary electrophoresis system fitted with a 64.5 cm x 50 μm internal diameter extended light path uncoated fused silica capillary with an effective length of 56 cm to the detector (Hewlett–Packard Co., Waldronn, Germany). The separations were performed at +25 kV and at 28°C using the buffer described above. The compounds were loaded under pressure (250 mbar s). Nicotinic acid was detected at 254 nm. The capillary was flushed with running buffer for 2 min between runs. Electropherograms were recorded on a Hewlett–Packard Ultra VGA 1280 computer using the Hewlett–Packard Chemstation software from which UV spectra and peak purity data were obtained. Peak areas were used in the calculations.

An Isco Model 3140 operating with the conditions described by Ward and Trenerry (1997) was used to determine the levels of niacin in fruit and vegetables after alkali extraction.

2.5.2. HPLC

Analyses were performed with a model 600E HPLC pump, model 700 WISP and a model 996 photodiode array detector using a 4 μm C8 NOVAPAK Radial-PAK cartridge (8 × 100 mm) equipped with a C18 precolumn (Waters Corporation, Milford, MA, USA) using a mobile phase consisting of a 15% methanol, 85% deionised water mixture containing 0.005M PIC A Reagent. The eluent flow rate was 1.5 ml/min. Nicotinic acid was detected at 254 nm. Peak areas obtained from a Waters Millennium data system were used in the calculations.

3. Results and discussion

The traditional AOAC colorimetric procedure using cyanogen bromide and sulphanilic acid (Cunniff, 1997a) and the microbiological assay using *Lactobacillus plantarum* (Ball, 1994; Cunniff, 1997b) both use an acidic extraction to liberate niacin from foods and vitamin preparations. This treatment liberates the nicotinamide from its coenzyme forms and simultaneously hydrolyses it to nicotinic acid. This treatment does not completely liberate the bound niacin from cereal products; alkali hydrolysis is necessary to achieve this. The AOAC colorimetric procedure is unreliable in certain circumstances and the microbiological assay time consuming (Kwiatkowska, Finglas, & Faulks, 1989). In some instances, HPLC provides a rapid, reproducible and accurate determinative step for the determination of niacin after acid extraction (Rees, 1989; van Niekirk, Smit, Strydom, & Armbruster, 1984; Vidal-Valverde & Reche, 1991). Other HPLC methods include the determination of niacin in meat (Takatsuki, Suzuki, Sato, Sakai, & Ushizawa, 1987; Tyler & Genzale, 1990), cereals (Tyler & Shrago, 1980) and coffee (Trugo, Macrae, & Trugo, 1985), but these use either an aqueous or alkali extraction to liberate the niacin from the matrix.

Chromatographic and electrophoretic procedures add an extra level of confidence to the quality of analytical measurements, especially if a photodiode array detector is used in the determination (Heiger, Kaltenbach, & Seivert, 1994; Vandepeer, Trenerry, & Keogh, 1996). The CE methods are robust, faster and more cost effective than methods based on HPLC. CE separations are characterised by the speed and excellent resolution of the separations. However, two of the major disadvantages of the technique are the lack of sensitivity compared to HPLC and the potential non-compatibility of the sample solutions with the buffers used for the determinations (Altria, Kelly, & Clark, 1996). The approach used to overcome these problems for the determination of niacin using an alkali extraction was to trap the nicotinic acid on a cation exchange SPE cartridge, remove unwanted interfering compounds by washing the column with methanol and then elute the nicotinic acid with a 2% solution of ammonia in methanol. Removal of the solvent and redissolving the residue in water provided an extract in a solvent that is more compatible with the running buffer (Ward, Trenerry, & Pant, 1996; Ward & Trenerry, 1997). This procedure also allows for a concentration step to be incorporated into the method, thus permitting much lower levels of niacin to be determined.

3.1. Hydrolysis of nicotinamide to nicotinic acid

The recommended procedure for extracting niacin from all food types is autoclaving the food in the presence of 1 M sulphuric acid for 30 min at 121°C. Milder conditions are also described in which non cereal foods were autoclaved with 0.5 M sulphuric acid for 30 min at 121°C. During autoclaving the nicotinamide is liberated from its co-enzymes and simultaneously hydrolysed to nicotinic acid (Ball, 1994). Preliminary experiments in our laboratory suggested that more vigorous conditions were necessary to completely hydrolyse nicotinamide to nicotinic acid. Complete hydrolysis of nicotinamide was achieved by either autoclaving in the presence of 0.8 M sulphuric acid for 2 h at 121°C or by heating the solution in a boiling water bath for 5 h. For practical purposes, autoclaving the acidic extracts at 121°C for 2 h was adopted as the preferred procedure.

3.2. Determination of niacin in foods

The successful use of SPE as a clean-up/ concentration step in determining niacin in a variety of foods after

alkali extraction suggested that a similar approach could be used to provide a suitable solution for CE analysis after an acidic extraction. For alkali extraction, the excess calcium hydroxide was removed from the filtered extract by the careful addition of saturated oxalic acid solution to a pH of 7. The insoluble calcium oxalate was easily removed by centrifugation. The SPE procedure also allows for a concentration step to be incorporated into the method, thus permitting much lower levels of niacin to be determined. In a similar fashion, excess sulphuric acid could be removed from the extract by the addition of saturated barium hydroxide solution to pH 7. Removal of the insoluble barium sulphate would then provide a suitable solution for SPE clean-up.

The proposed method was trialed on a limited range of meat and fish samples. The homogenised sample was mixed with 25 ml 0.8 M sulphuric acid and autoclaved for 2 h at 121°C (approx. 104 kPa). The solution was diluted with water, mixed thoroughly and centrifuged. An aliquot of the supernatant was adjusted to pH 7 with saturated barium hydroxide solution and made to volume, mixed and filtered. An aliquot was then passed through the activated C18 and SCX columns as previously described (Ward & Trenerry, 1997). The final solutions were analysed by CE and by HPLC. The CE buffer varied slightly from that used for the previous work, in that 7.5% acetonitrile was used as the organic modifier instead of 15% acetonitrile (Ward & Trenerry, 1997). The electropherograms from the acidic extraction were cleaner than those generated from the previous work. Baseline resolution of the niacin peak from other peaks in the electropherograms was evident in all of the samples. The results were encouraging, even though there was a slight difference between the levels determined by CE and by HPLC. The data are presented in Table 1. Saccharin was used as the internal standard for CE analyses. All sample solutions were also analysed without internal standard to ensure that there were no interfering compounds co-migrating with the internal standard. UV spectra and peak purity data collected by the photodiode array detectors indicated the nicotinic acid peak to be pure and free from interfering compounds (e.g., turkey mince, 998 out of a possible 1000; beef mince, 997 out of a possible 1000; raw salmon, 999 out of a possible 1000). The levels of niacin determined by this procedure were similar to those reported by English and Lewis (1991) (beef mince, 4.2 mg/100 g, canned tuna 7.8 mg/100 g). The SPE clean-up was attempted without neutralisation with saturated barium hydroxide solution with limited success. There was considerable variation between duplicate analyses, even though a respectable recovery on a spiked sample blank extract was achieved (122%). A more extensive range of meats (cooked and uncooked) were then analysed. The levels of niacin determined by CE and by HPLC are shown in Table 2. In all cases, there were no compounds interfering with the internal standard peak in the CE analyses, and the purity of the nicotinic acid peaks was confirmed by the UV spectra and peak purity data collected from the photodiode array detector (e.g. cooked lamb, peak purity 994 out of a possible 1000; raw lamb, 943 out of a possible 1000). A level of reporting of 0.5 mg/100 g could be achieved using these conditions. This was based on the signal to noise ratio of 3 for the peak corresponding to the lowest standard concentration used in the determination. Electropherograms and chromatograms of spam and cooked chicken extracts are displayed in Figs. 1 and 2, respectively.

The CE migration time of nicotinic acid was similar to the HPLC retention time (10 min), however, the overall CE run times were much shorter, due to the late eluting peaks with HPLC. The CE traces were also much cleaner. The CE instrument repeatability data (CV) for area calculation for seven repeat injections of chicken extract was 0.1%. Similarly, the instrument repeatability data (CV) for the variation in the migration time of nicotinic acid, compared to the variation of the migration time of the internal standard was 0.1%.

Table 1
Niacin levels determined after acid extraction by both CE and HPLC for a limited range of meat and fish samples (recoveries of niacin added to the samples prior to extracting with aqueous sulphuric acid are shown)

Sample	CE mg/100 g	CE (recovery %)	HPLC mg/100 g	HPLC (recovery %)
Turkey mince	5.3	106	4.8	115
Beef mince	3.9	88	3.8	107
Smoked chicken	3.6	–	3.2	–
Raw salmon	6.9	104	6.7	109
Raw tuna	8.6	112	9.1	119

– indicates no recovery data available.

Table 2
Niacin levels determined after acid extraction by both CE and HPLC for a variety of raw and cooked meats (recoveries of niacin added to the samples prior to extracting with aqueous sulphuric acid are shown)

Sample		CE (mg/100 g)	CE (recovery %)	HPLC (mg/100 g)
Spam		3.4	107	3.3
Chicken	Raw	6.0	101	5.5
	Cooked	8.0	111	7.5
Beef	Raw	3.7	105	3.4
	Cooked	4.9	97	4.3
Lamb	Raw	7.7	106	7.7
	Cooked	7.6	102	7.6
Pork	Raw	10.0	80	8.8
	Cooked	11.6	121	10.2
Fish	Raw	1.1	100	0.9
	Cooked	1.7	104	1.5

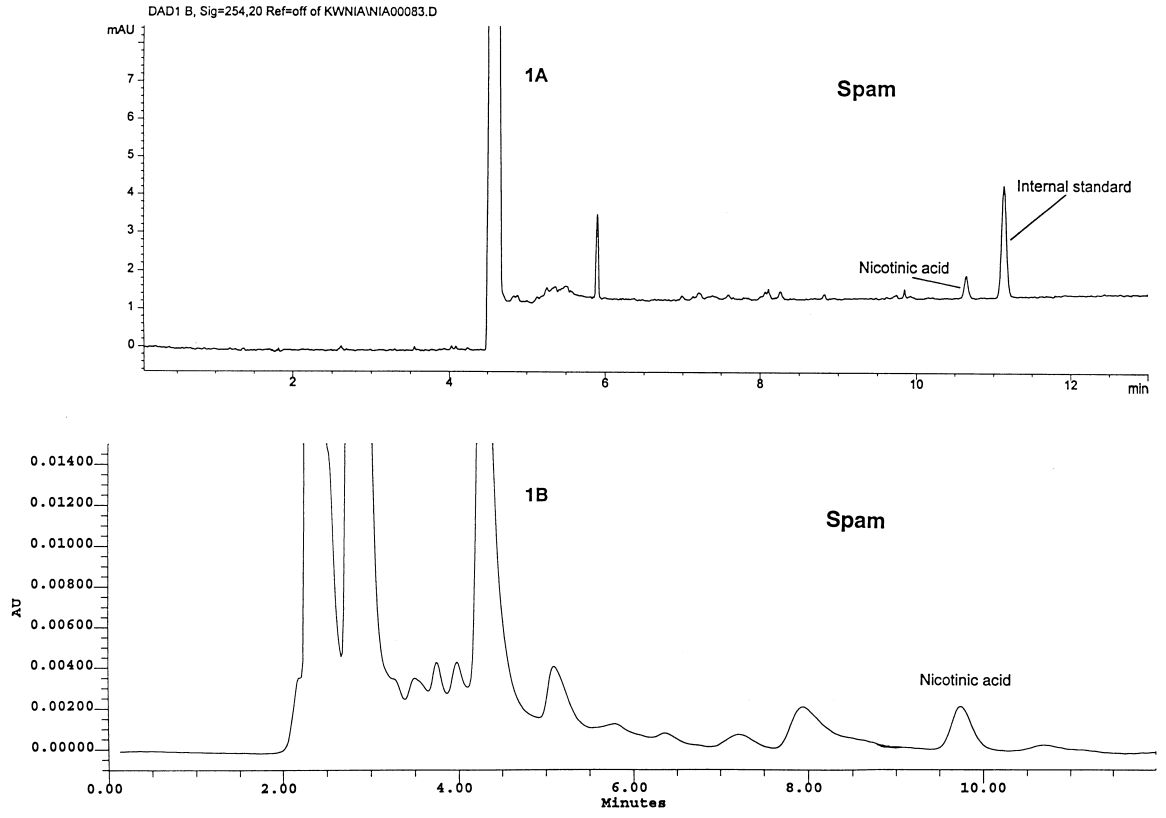

Fig. 1. (A) Electropherogram and (B) partial chromatogram of spam (canned ham) containing 3.4 mg/100 g of niacin after acid extraction and SPE cleanup using the conditions described in the Experimental section.

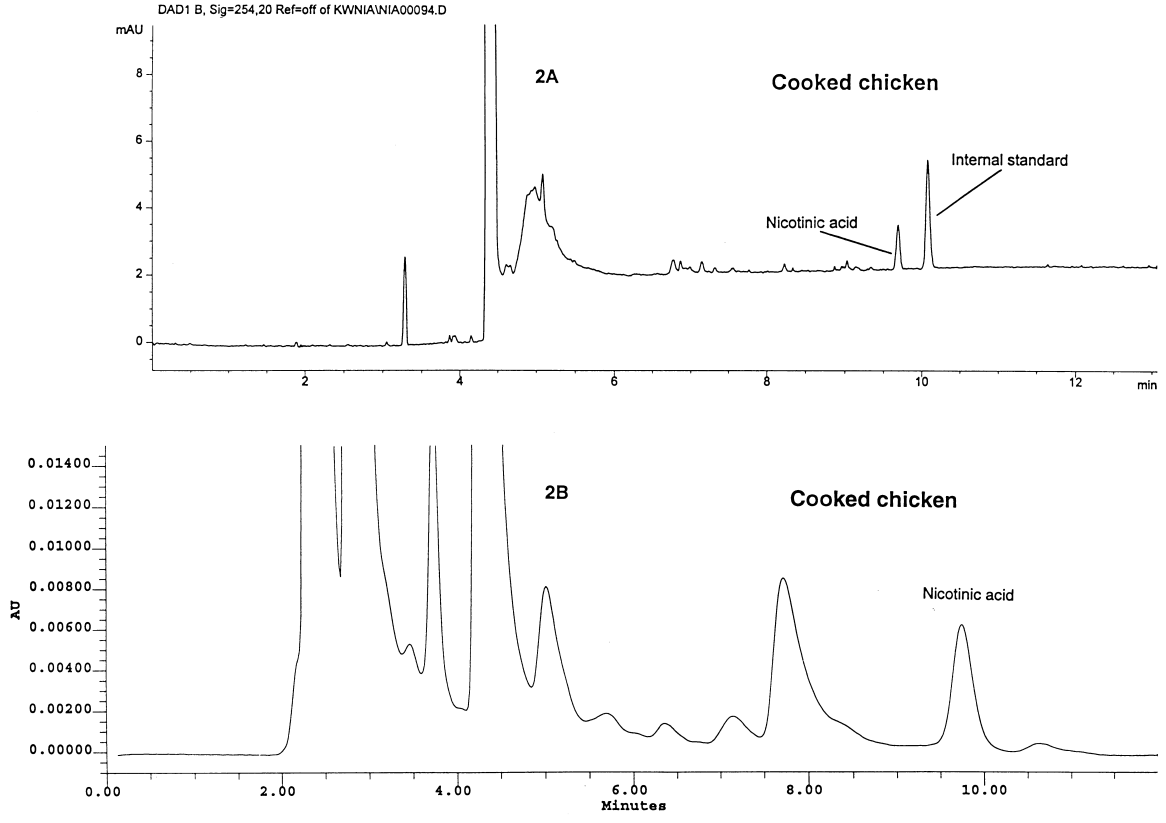

Fig. 2. (A) Electropherogram and (B) partial chromatogram of cooked chicken containing 8 mg/100 g of niacin after acid extraction and SPE cleanup using the conditions described in the Experimental section.

Seven portions of spam (canned ham) were analysed in one batch to determine the overall accuracy/repeatability of the method. The levels of niacin varied between 3.3 and 3.7 mg/100 g giving a mean value of 3.5 mg/100 g and a CV for the procedure of 4.7%. The levels of niacin in the foods were also similar to the values reported by English and Lewis (1991) and by Greenfield (1987). No direct comparison of the results can be made as different samples were used to produce the analytical results. The UV spectra and the peak purity data of the peaks corresponding to nicotinic acid in the electropherograms and chromatograms confirmed the integrity of the peaks.

Table 3
Niacin levels determined by both CE and HPLC for a other foods (recoveries of niacin added to the samples prior to extracting with aqueous sulphuric acid are shown)

Sample	CE (mg/100 g)	HPLC (mg/100 g)	CE (recovery %)	English and Lewis (1991)
Potato	1.1	1.0	74	–
Potato peeled	0.9	1.0	80	1.3
Broccoli	0.6	< 0.2	–	0.5
Banana	0.4	0.3	100	0.5
Orange	0.2	< 0.2	80	0.3
Cow's milk	< 0.5	< 0.5	84	< 0.2
Soy milk	< 0.5	< 0.5	95	< 0.2
Cashew nut	0.9	1.0	99	1.8
Almond	3.1	2.2	107	3.9
Yeast	35	38	35	–

– indicates that no comparable data are available.

The method was then applied to samples of potato, broccoli, banana, orange, yeast, cashew nut, almond, cow's milk and soy milk. The levels of niacin in these foods after acid extraction and SPE clean-up are listed in Table 3. The electropherogram and chromatogram of a potato extract is seen in Fig. 3. A 3 g sample of the fruit was extracted to compensate for the lower values of niacin expected in the samples. This also resulted in a lower level of reporting of 0.2 mg/100 g. 1 g SPE cartridges were also used to assist in the clean-up of the samples. Previous unpublished work by the authors using an alkali extraction (Ward & Trenerry, 1997) to determine niacin in fruits and vegetables indicated that the larger SPE columns were necessary to achieve reproducible results.

The procedure was suitable for potato, banana, orange, cashew nut, cow's milk and soy milk. The levels of niacin in potato, orange, banana and cashew nut were quite low and below the level of reporting for the cow's milk and soy milk. The recoveries of niacin added to these samples prior to analysis were in the

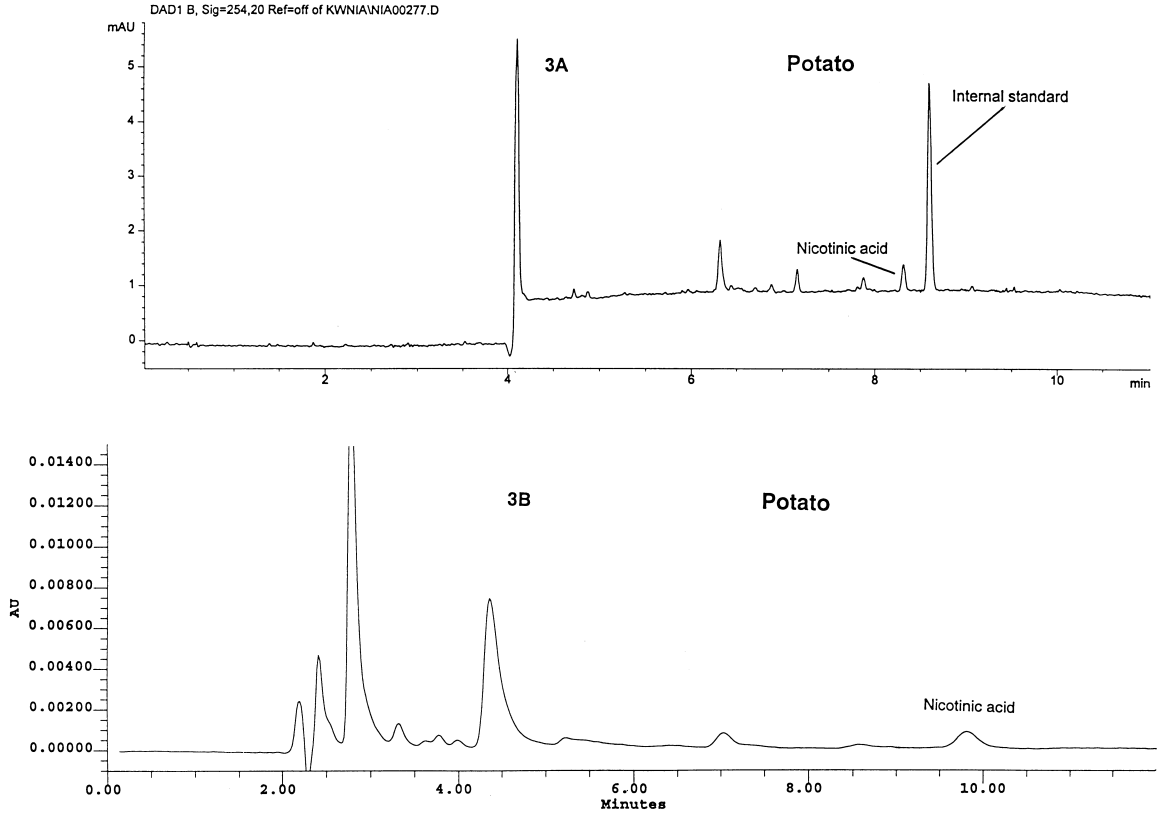

Fig. 3. (A) Electropherogram and (B) partial chromatogram of potato containing 1.1 mg/100 g of niacin after acid extraction and SPE cleanup using the conditions described in the Experimental section.

Table 4
Niacin levels determined after alkali extraction and determined by CE for a variety of raw and cooked vegetables and fruits (recoveries of niacin added to the samples prior to extracting with aqueous calcium hydroxide are shown)

Sample		CE (mg/100 g)	Recovery %
Vegetables			
Broccoli	Raw	0.6	92
	Cooked	1.0	104
Potato	Raw	0.8	91
	Cooked	0.8	88
Pumpkin	Raw	< 0.5	95
	Cooked	1.1	84
Corn	Raw	1.3	92
	Cooked	1.3	87
Beans	Raw	< 0.5	91
	Cooked	0.6	90
Peas	Raw	1.9	82
	Cooked	2.4	86
Fruit			
Apple		< 0.2	104
Orange		0.4	109
Grape		< 0.2	89
Kiwi fruit		0.3	101
Banana		0.5	87
Strawberry		0.3	98
Canned fruit salad		0.3	120
Rockmelon		0.5	98
Honeydew melon		0.2	107
Tomato		0.3	93
Tomato sauce		0.6	104

Table 5
Comparison of the niacin levels in meat and cereal products after acid and alkali extraction determined by both CE and HPLC

Sample		CE (mg/100 g)		HPLC (mg/100 g)	
		Acid extract	Alkali extract	Acid extract	Alkali extract
Meat					
Chicken	Raw	6.0	5.5	5.5	5.5
	Cooked	8.0	8.4	7.5	7.9
Beef	Raw	3.7	3.5	3.4	3.4
	Cooked	4.9	4.8	4.3	4.2
Lamb	Raw	7.7	7.6	7.7	7.0
	Cooked	7.6	9.0	7.6	8.1
Pork	Raw	10.0	8.3	8.8	7.9
	Cooked	11.6	10.5	10.2	10.2
Fish	Raw	1.1	0.9	0.9	0.8
	Cooked	1.7	1.5	1.5	1.4
Cereals					
VMA 195		16.3	16.6	–	–
Wheat bran		15.2	17.6	–	–
Breakfast cereal		4.1	4.4	–	–
Wholemeal bread		4.2	4.6	–	–
White bread		2.3	1.7	–	–
Oats		1.7	1.6	–	–
White rice		< 0.5	< 0.5	–	–

– indicates that the data are not available.

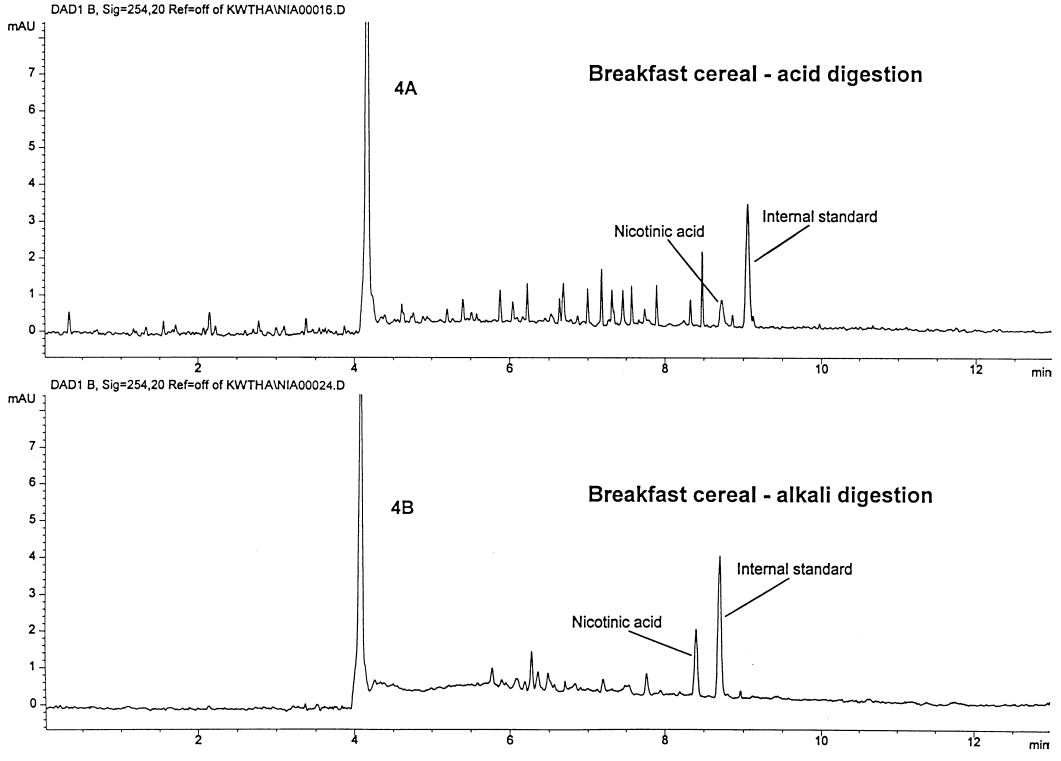

Fig. 4. Electropherograms showing breakfast cereal, (A) after acid hydrolysis and SPE cleanup and (B) after alkali extraction and SPE cleanup using the conditions described in the Experimental section.

range of 90–100%. Poor duplicate analyses, coupled with unsatisfactory recoveries and complex electropherograms indicated that the method was unsuitable for yeast and almond.

The levels of niacin in fruit and vegetables were more readily determined by using the alkali extraction procedure described by Ward & Trenerry (1997). The sample extracts were analysed by CE. The levels of niacin in the foods were similar to the values reported by English and Lewis (1991). In all cases, nicotinic acid was well separated from the other peaks in the electropherograms. UV spectra and peak purity data confirmed the identity of the nicotinic acid peak. Good recoveries (mean 95%, range 84–120%, $n = 23$) of nicotinic acid added prior to extraction were achieved. The data are presented in Table 4.

3.3. Comparison of acid and alkali extraction

A number of samples were analysed concurrently by both acid and alkali extraction. The levels determined by CE and HPLC analysis are displayed in Table 5. The results indicate that acid and alkali extraction give similar levels of niacin in the foods. Slightly higher levels are found in the meat samples with acid extraction, and higher levels in the cereal products by alkali extraction. No real comparison can be made with the fruit and vegetables as reliable results were only available from the alkali extraction. Electropherograms of a breakfast cereal after acid and alkali extraction are displayed in Fig. 4. A similar trend was observed by Van Niekirk et al. (1984) when the levels of niacin determined in cereals and mushrooms were determined by both acid and alkali extraction and HPLC analysis.

4. Conclusion

A robust method for the determination of niacin in raw and cooked meat and fish samples has been developed. The niacin is extracted from the samples with 0.8 M sulphuric acid, followed by solid phase extraction to isolate and concentrate the liberated nicotinic acid. CE and HPLC were used as the determinative steps. The method was suitable for some other foods, but more reliable results for fruit and vegetables were obtained using an alkali extraction. The sample extracts can be analysed by either CE or by HPLC, however CE is preferred as it produces cleaner traces, is faster and is more cost effective.

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