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Determination of free calcium in vegetables by capillary zone electrophoresis

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Abstract

A capillary zone electrophoretic method was developed for the determination of free calcium (calcium ions) in vegetables. Calcium ions (Ca^{2+}) were extracted by boiling crushed vegetables in water for 15–20 min and determined directly by capillary zone electrophoresis based on complexation with ethylenediaminetetraacetic acid. The relative standard deviations of peak area and migration time of calcium ion were 2.9 and 0.4%, respectively. The recovery of calcium ions was 96–111%. Calcium ion in various vegetables was determined by the method outlined. The results agreed with those obtained by capillary isotachophoresis. The method was also applied for the determination of calcium ions in vegetables cultivated using fermented blue mussels to examine the usefulness of fermented blue mussels as a fertilizer for vegetables.

Keywords: Vegetables; Food analysis; Calcium

1. Introduction

It is well-known that calcium intake from foods is closely related to physiological effects in the human body [1]. Considerable amounts of calcium are contained in vegetables although major calcium sources are milk and small fish with edible bone etc. [2]. It is, therefore, important to determine calcium levels in vegetables.

Calcium exists in vegetables in the form of free calcium (calcium ion), calcium oxalate and other calcium compounds [2]; it is considered that the oxalate depresses the absorption of calcium and calcium bioavailability [3]. Total calcium in vegetables has been determined by titration using potassium permanganate [4] and ethylenediaminetetraacetic acid (EDTA) [4] and by atomic absorption spectrometry (AAS) [5]. Various metals including calcium were determined in radish using flame emission spectrometry and inductively coupled plasma atomic emission spectrometry from the viewpoint of environmental chemistry [6]. AAS and ion chromatography were used for the characteristic determination of calcium in vegetables [2] and sesame seeds [3].

Recently, capillary zone electrophoresis (CZE) has been used for the determination of magnesium and calcium ions in wheat flour [7], nitrate and nitrite ions in pickles and processed foods [8], and the amino acid composition of gluten in the processing

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of bread [9]. In our previous paper [10], CZE using complexation with EDTA was proposed for the determination of magnesium and calcium ions in seawater. In the present paper, utilizing this method, optimum analytical conditions were established for the determination of calcium ion in vegetables. Calcium ion in various vegetables was determined by the method and the results were compared to those obtained by capillary isotachophoresis (cITP).

In addition, blue mussels, living in large numbers on the vertical walls in the closed sea, take in suspended organic matter from seawater and are utilized for their ability to clean seawater [11]. The usefulness of blue mussels as a fertilizer was examined. Chinese vegetables were cultivated using fermented blue mussels; the calcium ion content in the vegetables, determined by the proposed method, was considered as an index indicating their usefulness as a fertilizer.

2. Experimental

2.1. Apparatus

2.1.1. CZE

A Perkin-Elmer (Foster City, CA, USA) Model 270A capillary electrophoretic analyser was used with a UV–Vis absorbance detector. A polyimide-coated fused-silica capillary, 50 μm I.D.×375 μm O.D., served as the capillary electrophoresis column. The total length of the column was 72 cm; the effective length was 25 cm. Peak area and height were measured using Hitachi (Tokyo, Japan) Model D-2500 Chromato-Integrator.

2.1.2. cITP

A Shimadzu (Kyoto, Japan) Model IP-2A isotachophoretic analyser was used with potential-gradient detector. The main column was a fluorinated ethylene–propylene copolymer tube (15 cm×0.5 mm I.D.), and the precolumn was a polytetrafluoroethylene tube (15 cm×1.0 mm I.D.). A Hamilton (Reno, NV, USA) 1701-N microsyringe was used for the injection of samples into the isotachophoretic analyser. A Unicam Analytical Systems (Cambridge, UK) PW9421 pH meter was used.

2.2. Reagents

All reagents were of analytical-reagent grade and used as received. Distilled, demineralized water was used throughout. Sodium tetraborate and tris(hydroxymethyl)aminomethane (Tris) were obtained from Nacalai Tesque (Kyoto, Japan). EDTA disodium salt and hydroxypropylmethylcellulose (HPMC) were obtained from Ishidzu Pharmaceutical (Osaka, Japan) and Aldrich (Milwaukee, WI, USA), respectively. Sodium hexanoate was obtained from the Tokyo Chemical Industry (Tokyo, Japan). Standard solutions of calcium ion were prepared by dissolving calcium chloride (Kishida-Kagaku, Osaka, Japan) in pure water obtained from a Yamato-Kagaku (Tokyo, Japan) Model WG220 automatic still and a Nihon Millipore (Tokyo, Japan) Milli-Q II system.

2.3. Procedure

2.3.1. Sample preparation

Calcium ion was extracted from vegetables using a modification of the procedure proposed by Ishii and Takiyama [2]. A 1-g weight of crushed vegetables in a porcelain mortar was weighed into 50 ml of boiled water (in a 100-ml tall beaker) and boiling was allowed to continue for 15–20 min. Whole leaf including leaf blade and leafstalk was used for the sample. The beaker was cooled with tap-water. The contents were filtered through a 0.45-μm membrane filter. The total volume of the filtrate was adjusted to 100 ml with water. The resulting solution was injected into the capillary electrophoretic and isotachophoretic analysers.

2.3.2. Analysis by CZE

All solutions, including a buffer solution, were filtered through a 0.45-μm membrane filter before use. The detection wavelength was set at 200 nm. The thermostat was maintained at 35°C. The capillary was filled with the buffer solution (20 m*M* sodium tetraborate containing 2.0 m*M* EDTA, pH 9.2) by vacuum for 3 min. A small amount of the sample solution (12 nl) was injected into the capillary electrophoretic analyser by vacuum for 3 s. The volume of material injected per unit time (V_t, nl/s) is determined by the following equation [12]:

$$V_t = \frac{\Delta P D^4 \pi}{128 \eta L} \quad (1)$$

where ΔP equals the pressure drop, D is the capillary internal diameter, η is the viscosity, and L is the length of the capillary. A charge of +20 kV was applied with the sample inlet side being set as anode. Each step was automatically run. A calibration graph was prepared by applying the method to synthetic standards.

2.3.3. Analysis by cITP

A 10-μl portion of the sample solution was injected into the isotachophoretic analyser. The migration current was maintained at 200 μA for the first 8 min and then reduced to 50 μA. The leading electrolyte was an aqueous solution containing 5 m*M* hydrochloric acid and 0.1% (w/w) HPMC; the pH was adjusted to 8.5 with Tris. The terminating electrolyte was 10 m*M* sodium hexanoate solution containing 0.5 m*M* EDTA.

3. Results and discussion

3.1. Pretreatment method of vegetables

In general, juicy vegetables are crushed in a porcelain mortar etc. to prepare homogeneous samples [13]. The effect of the pretreatment method on the analytical results for calcium ion was examined. Commercially available chingentsuais, a kind of Chinese vegetable, were chopped up or crushed in a porcelain mortar; calcium ion in the samples was extracted according to the sample preparation procedure described above. Concentrations of calcium ion were determined by CZE; the amount of calcium ion in 100 g of a chingentsuai was obtained. Four stumps of chingentsuais purchased at different dates were analysed. The concentrations of calcium ion in crushed samples were 13–19% higher than those in chopped-up samples except for sample 2, as evident in Table 1. Thus, crushing was adopted as the pretreatment method of vegetables.

When the boiling time was varied in the range 5–30 min, the peak area for calcium ion was almost constant. However, the rate of filtration was slow at a boiling time of 10 min or shorter, so 15–20 min was adopted as the boiling time.

Table 1
Effect of pretreatment method of vegetables on analytical results for calcium ion

Sample[a]	Ca^{2+} (mg/100 g)	
	Crush	Chop up
1	95	83
2	61	61
3	57	48
4	61	54

[a] Sample, commercially available chingentsuais.

3.2. Determination of calcium ion in various vegetables

3.2.1. Analysis by CZE

A calibration graph for calcium ion was linear when using peak area, while it was curved using peak height up to 25 mg/l. Peak area was, therefore, used for the calculation of the concentration of calcium ion in vegetables. The regression equation relating area response (y: arbitrary units) to concentration (x: 0–25 mg/l) was $y = 2644x + 274$ (correlation coefficient 0.9999) for calcium ion. Limit of detection for calcium ion was 0.26 mg/l [10]. A commercially available chingentsuai was analysed with four replicates to examine the precision of the method. The relative standard deviations of the peak area and the migration time were 2.9 and 0.4%, respectively. Chingentsuai samples, with 5.0–20.0 mg/l of calcium ion added, were analysed by the method. The recovery of calcium ion was 96–111%, as shown in Table 2.

The proposed method was applied to the determination of calcium ion in various vegetables

Table 2
Recovery of calcium ion in vegetables by the proposed method

Ca^{2+}

Added (mg/l)	Found (mg/l)	Recovery (%)
-	6.1	-
5.0	10.9	96
10.0	16.3	102
15.0	22.8	111
20.0	27.7	108

Sample: commercially available chingentsuai.

Table 3
Results for calcium ion in various vegetables

Sample	Ca^{2+} (mg/100 g)		Total Ca[a] (mg/100 g)
	CZE	cITP	
Chingentsuai	105	108	130
Komatsuna	144	153	290
Spinach	21	32	55
Perilla	117	129	220
Garland	24	36	90

[a] Cited from Ref. [14].

found on the market. The results are shown in Table 3; komatsuna is a kind of Japanese vegetable. The concentrations of calcium ion in the vegetables were 27–81% of their total calcium concentrations [14]. The correlation coefficient between the concentrations of calcium ion and those of total calcium was 0.9169 in the vegetables. The concentrations of calcium ion in the spinach and perilla were higher than those reported by Ishii and Takiyama [2]; crushing was not adopted in their pretreatment method. It is thought that the differences result partly from the difference between our pretreatment method and their method. It was necessary to remove proteins from the extract prior to the ion chromatographic determination [2], but it was not in the CZE analysis.

3.2.2. Analysis by cITP

Nakabayashi and his coworkers [15] investigated the isotachophoretic separation of metal ions, with a polyaminopolycarboxylic acid as both the terminating ion and the complex agent. Hine [16,17] proposed the use of the terminating electrolyte containing chelating agents for the isotachophoretic analysis of metal ions. This method was adopted and modified for the selective determination of calcium ion. Authors examined the pH of the leading electrolyte for the determination of calcium ion using a terminating electrolyte containing EDTA. Calcium ion was completely separated from magnesium ion with the leading electrolyte adjusted to pH 8.5, whereas it was not with a leading electrolyte adjusted to pH 6.0. A calibration graph for calcium ion was linear up to 25 mg/l. The regression equation relating zone-length response (y: mm) to concentration (x: 0–25 mg/l) for calcium ion was $y = 1.43x + 0.5$ (correlation coefficient 0.9991). The recording speed was adjusted to 40 mm/min. The results are shown in Table 3. An isotachopherogram of the komatsuna sample is shown in Fig. 1. The results obtained by the CZE analysis agreed closely with those obtained by the cITP analysis (correlation coefficient, 0.9980).

3.3. Usefulness of blue mussels as fertilizer

It is necessary to remove blue mussels from vertical walls to utilize them for preventing eutrophication in the closed sea. Otherwise, seawater is contaminated with dead blue mussels from summer to autumn [11]. If collected blue mussels are allowed to stand, they putrefy and give out a bad smell. In contrast, mussels contain protein (10 300 mg/100 g), phosphorus (160 mg/100 g), potassium (230 mg/100 g) and calcium (43 mg/100 g), which are important fertilizer components [18,19]. Therefore, the collected blue mussels were fermented using commercially available EM (Effective Micro-organisms) [20] to depress the stench; chingentsuais were cultivated using the fermented blue mussels as fertilizer. The concentrations of calcium ion in the chingentsuais were determined after harvesting by the CZE method. The calcium ion contents in the

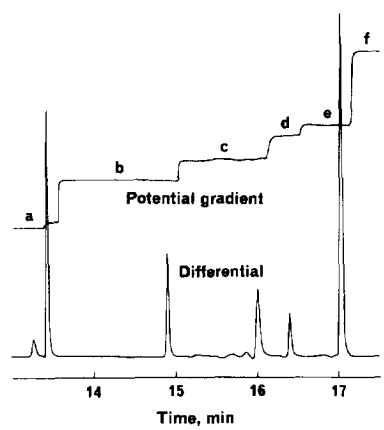

Fig. 1. Isotachopherogram of a komatsuna treated by the proposed method: (a) Cl^-; (b) EDTA; (c) HCO_3^-; (d) Mg(II)-EDTA; (e) Ca(II)-EDTA; (f) $C_5H_{11}COO^-$.

Table 4
Calcium ion contents and weight of chingentsuais

Chingentsuai	Ca^{2+} (mg/100 g)	Mass (g)
A	146	113
B	105	110
C	123	143
D	136	139

A: cultivated in soil containing a layer of the blue mussels fermented with EM; B: cultivated in a mixture of soil and blue mussels fermented with EM; C: cultivated in soil containing a layer of crushed blue mussels; D: purchased.

cultivated chingentsuais and the mass of these chingentsuais were about the same as those for the purchased one, as shown in Table 4. An electropherogram of chingentsuai A in Table 4 treated by the method is shown in Fig. 2.

The proposed method is simple, rapid and possesses sufficient detection power and precision to be useful for the determination of calcium ion in vegetables. It was found that blue mussels were useful as a fertilizer for vegetables.

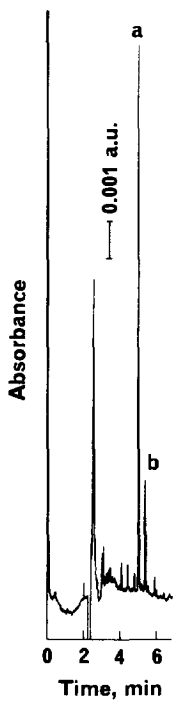

Fig. 2. Electropherogram of a chingentsuai treated by the proposed method; sample, chingentsuai A in Table 4; (a) Ca(II)-EDTA; (b) Mg(II)-EDTA.

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