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Nitrate-N determination in leafy vegetables: Study of the effects of cooking and freezing

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Abstract

Nitrate upon reduction to nitrite can cause methaemoglobinaemia or act as precursor in the endogenous formation of carcinogenic nitrosamines. The leafy vegetables are the major vehicle for the entry of nitrate into the human system. The present study was conducted to establish a flow injection analysis (FIA) technique to investigate the nitrate-N contents of four commonly consumed fresh leafy vegetables (Chinese cabbage, celery, lettuce and English cabbage) from market in Fiji. Two extraction techniques (activated carbon and alkaline extraction) were assessed to extract nitrate-N and the activated carbon extraction was preferred over alkaline extraction and applied. The recoveries of spiked nitrate-N in vegetable matrices ranged from 90.40% to 112.80% in activated carbon extraction with an average of 100.62%. The effects of cooking (boiling, baking and frying) and deep-freezing on the nitrate-N contents were also studied. Nitrate contents in selected leafy vegetables were determined by FIA coupled with Greiss protocol involving sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride as color reagents. Nitrate was determined in the linear range from 1.0 to 20.0 mg L^{-1} with the method detection limit of 0.042 mg L^{-1} (0.34 mg kg^{-1}). The results of the study show that nitrate contents in fresh leafy vegetables ranged from 1297 to 5658 mg kg^{-1}. Boiling reduces nitrate content by 47–56% whereas frying in Soya bean oil elevates nitrate content by as much as 159–307%. No significant change was observed in nitrate content after baking. The deep-freezing of the selected leafy vegetables shows that nitrate-N content fluctuates slightly from the original nitrate-N values over the seven day period. The FIA throughput was 38 samples h^{-1}.

Keywords: Nitrate; Nitrate in vegetables; Leafy vegetables; Flow injection analysis; Fiji leafy vegetables; Fiji

1. Introduction

Vegetables play an important role in human nutrition since they are an outstanding source of vitamins, minerals and biologically active compounds (Kmiecik, Lisiewska, & Slupski, 2004). Consequently, vegetables are high-value crops and provide a consistent income for vegetable farmers in Fiji. Nitrates are present naturally in most fruits and vegetables. Human nitrate intake is mainly from vegetables, water supplies and from additives/preservatives used in meat (Tilbury, 1980; Wolff & Wasserman, 1972). About 87% of the total nitrate concentration in a normal diet is believed to be a direct result of vegetable intake (Huarte-Mendicoa, Astiasaran, & Bello, 1997).

Nitrates form part of the essential chemistry of soils and plants. Thus plant roots are able to absorb nitrate directly from the soil. Nitrate is the ionic form, which supplements the essential plant nutrient, nitrogen (Cieslik & Sikora, 1998). Since nitrogen plays a key role in plant growth, the most readily available agricultural fertilizers contain nitrate. It has been discovered that green leafy vegetables (such as lettuce, spinach, beets, radishes, celery, etc.) contain the highest levels of nitrates (MAFF, 1998; Wolff & Wasserman, 1972). Nitrate contamination in vegetables occurs when crops absorb more than they require for their sustainable growth. Spinach, lettuce, broccoli, cabbage,

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celery, radish, beetroot, etc. possess the tendency to accumulate nitrates. On the other hand, vegetables such as carrots, cauliflower, French beans, peas and potatoes seldom accumulate nitrates. Nitrate content of vegetables may range from 1 to 10,000 mg kg^{-1} (Ximenes, Rath, & Reyes, 2000).

The main concern for the public health is the link between nitrates and stomach cancer. It is due to the fact that nitrates help in the formation of carcinogenic nitrosamines. The elevation of gastric pH > 5.5 leads to bacterial growth followed by rapid conversion of nitrate to nitrite. Nitrite is a precursor in the formation of nitrosamines (Tannenbaum & Correa, 1985). Another important concern is that vegetables are an important part of most babies' diets (Huarte-Mendicoa et al., 1997). Young babies with low stomach acidity may suffer from infantile methemoglobinemia due to excessive nitrates in their diet, where nitrite is substituted for oxygen in hemoglobin and death may occur (Ezeagu, 1996; Gundimeda, Naidu, & Krishnaswamy, 1993; Swann, 1975). Even after such a high risk on public health there is no data available on Fiji's commonly consumed leafy vegetables. This forms the basis for the regular monitoring of nitrate levels in commonly consumed Fiji's fresh vegetables.

Nitrates in vegetables have neither taste nor smell but are identified using laboratory tests. Various methods have been developed to determine nitrates in food, water and other matrices. Over the years, analytical techniques, such as spectrophotometry (Huarte-Mendicoa et al., 1997; Nagaraja & Kumar, 2002), potentiometry (Perez-Olmos, Herrero, Lima, & Montenegro, 1997), ion chromatography (Mcmullen, Cassanova, Gross, & Schenck, 2005), polarography (Ximenes et al., 2000), capillary electrophoresis (Oztekin, Nutku, & Erim, 2002) as well as high-performance liquid chromatography (Reece & Hird, 2000; Sanderson, Rassconsaul, & Lee, 1991) have been used.

Despite the prevalent variations in techniques available, the common method for nitrate estimation involves quantitative reduction of nitrate to nitrite by spongy cadmium (Gundimeda et al., 1993). Nitrite undergoes diazotization and then determined by UV/VIS spectrophotometry. Despite the simplicity and easy applicability of this technique it is essentially time consuming, which makes it unsuitable for routine analysis of large numbers of samples. With the advent of automated continuous flow systems (flow injection analysis, FIA) based on colorimetry, the analysis of nitrates/nitrites has become much more time and labor efficient. A number of researchers have determined nitrate-N in different matrices using FIA (Andrade, Viana, Guadagnin, Reyes, & Rath, 2003; Higuchi & Motomizu, 1999; Lima, Rangel, & Souto, 1995; Monser, Sadok, Greenway, Shah, & Uglow, 2002). The present study is devoted to the optimization of a FIA method with photometric detection and is applied for the determination of the nitrate-N content in four commonly consumed fresh leafy vegetables marketed in Fiji. The effects of various cooking methods and deep-freezing on the nitrate-N contents in leafy vegetables have also been investigated.

2. Materials and methods

2.1. Fresh and frozen plant materials

Fresh leafy vegetables, *i.e.*, Chinese cabbage (*Brassica chinensis*), English cabbage (*Brassica oleracea* Var. capitata), celery (*Apium graveolens* L.) and lettuce (*Latuca sativa*) were collected from the Suva municipal market. Each type of vegetable was bought from six different vendors at random. After sampling each vegetable was rinsed with water to remove any soil or wind borne particles that may have been present on the vegetables. From the fresh vegetables, sub-samples were taken for the study of the effects of cooking on nitrate-N concentration. After cooking all sub-samples were labeled and frozen in acid pre-washed (snap-lock) plastic bags. All frozen samples were freeze-dried within 24 h. Freeze-drying times depended on water content of vegetables. After freeze-drying all samples were labeled and stored in acid pre-washed (snap-lock) plastic bags.

2.2. Reagents and standards

Unless specified otherwise all reagents used in this study were of analytical-reagent grade. The term "water" implies distilled de-ionized water (DDW, 18 MΩ cm^{-1}) and used for all sample extraction and vegetable sample preparation. All glassware used was soaked in 10% HCl for 24 h, and rinsed several times with DDW prior to use. The standard nitrate solutions were prepared by dissolving 0.25 g of KNO_3 (Biolab, Australia) in 250 mL of water, which gave rise to a 0.004 g mL^{-1} of stock solution. Serial dilutions were made to obtain the standard concentrations; 1.0, 4.0, 8.0, 12.0, 16.0 and 20.0 mg L^{-1} nitrate-N.

2.2.1. Sulfanilamide color reagent

Sulfanilamide color reagent was prepared in a 1 L volumetric flask, containing 600 mL of water, 25 mL of 85% phosphoric acid and dissolving 10 g of sulfanilamide (SA) (Ajax Finechem, Australia). *N*-(1-Naphthyl)ethylenediamine dihydrochloride (NED) (0.25 g) (BDH, England) was added to the solution. The mixture was stirred to dissolve for 30 min and diluted to 1 L. Resulting solution was stored in a dark amber color bottle and discarded when pink.

2.2.2. Ammonium chloride buffer

Ammonium chloride buffer was prepared in a 1 L volumetric flask by dissolving 21.25 g of ammonium chloride (Asia Pacific Specialty Chemicals Ltd., Australia) and 1.0 g of disodium ethylenediaminetetraacetic acid dihydrate (Sigma–Aldrich) in 800 mL of water. The mixture was diluted to 1 L while the pH adjusted to 8.5 with 10% NaOH (w/v).

2.3. Extraction and analysis

Two extraction techniques were tested on three types of leafy vegetables (Chinese cabbage, celery and lettuce). The most efficient technique was then employed throughout for the extraction of nitrate-N in this study.

2.3.1. Extraction via activated carbon

The extraction of nitrate-N using activated carbon was performed using Hunt and Seymore (1985) method. Freeze-dried vegetable sample (0.1–0.2 g) was added to 50 mL of water in a screw-capped bottle (120 mL capacity). Activated carbon (100–250 mg) was added and the bottle was tightly screw-capped. The mixture was then shaken for 30 min on a Stuart Scientific shaker SF1. A portion of the extract was filtered through a 0.45 μm membrane filter into a 50 mL polypropylene vial ready for analysis. The first 20 mL of the extract was discarded to prevent any residual contamination from the filter paper. If clear extracts were not obtained, the samples were centrifuged at 3000 rpm for 10 min.

2.3.2. Alkaline extraction

Sen and Donaldson (1978) method with minor modifications was used for the extraction of nitrate-N in the present study and for its comparison with the activated carbon extraction. Homogenized sample (10 g) was accurately weighed and blended for 5 min with 70 mL of water. Then 12 mL of 2% NaOH was added while pH *ca.* 8 was adjusted with 2% NaOH (avoiding excess NaOH). The slurry was transferred to a 200 mL volumetric flask and heated on water bath (50–60 °C) with occasional swirling until the temperature of the suspension reached about 50 °C. $ZnSO_4$ (10 mL) was added and temperature of the suspension maintained at about 50 °C for further 10 min. If a white precipitate of $Zn(OH)_2$ did not appear, 2–5 mL of 2% NaOH was added (avoiding excess NaOH). Contents were cooled to room temperature by immersing flask in cool water bath. The solution was diluted to a fixed volume with water and mixed thoroughly. Then the solution was filtered through a 0.45 μm membrane filter and collected in an Erlenmeyer flask. The solution was kept stoppered until analysis.

2.4. Cooking details

Boiling: All four leafy vegetables samples, having 24 sub-samples, were boiled separately for 10 min until cooked. After boiling all samples were drained on absorbent paper. *Baking:* All the representative vegetable samples were cleaned, pricked over their entire surface and baked at 180 °C for 25 min in an Omega mini kitchen oven. *Frying*: The vegetable samples were fried in Soya bean oil for 12 min. After frying samples were cooled, drained on absorbent paper and sliced. After cooking all the samples were labeled and frozen in acid pre-washed (snap-lock) plastic bags.

2.5. Deep-freezing

The four leafy vegetable samples, *i.e.*, 16 sub-samples, were purchased from the Suva municipal market from four different vendors at random. All samples were stored in a deep-freezer (−20 °C) until the day of analysis.

2.6. Flow system and apparatus

The extracted samples were analyzed using a Lachat QuickChem 8000 flow injection analyzer. A schematic diagram of the flow injection system employed for the quantification of nitrate-N is shown in Fig. 1. The manifold was equipped with a peristaltic pump, P, (RP-100 Series), an injection valve, V, a copperised cadmium reduction column, C, (Lachat part no. 50327) and a two state switching valve, SV. It was also equipped with a 60-position rack, an auto-sampler with sample volume of 200 μL, a 10 mm path length flow cell and a colorimetric detector, D, having UV filter of 520 nm. The teflon tubes R_1, R_2 and R_3 of 1.07 mm i.d. were used for the carrier water, buffer and reagent flow, respectively, while teflon tubes used for the manifold connections, the delay coil, DC (70 cm), and the reaction coil, RC (70 cm) were of 0.8 mm i.d.

All pH measurements were made using Hanna Instruments Microprocessor 8521 pH meter. The pH meter was standardized with the use of standard buffers (Aldrich). A Stuart Scientific shaker model SF1 was used for shaking all samples while extraction of nitrate-N was carried out.

2.7. Analytical procedure

The six calibration standards and extracted sample solutions were placed in sample tubes on the 60-position rack. The auto-sampler filled the solutions into injection valve (V) that was then injected into the carrier flow stream (R_1), which merged with the buffer (EDTA and NH_4Cl, pH 8.5) stream, R_2. The sample along with buffer progressed through the copperised cadmium (Cd/Cu) reduction column, C, reducing the nitrates to nitrites. The stream having nitrites merged with R_3, which contained color-developing reagent solution. At this junction the diazotization/coupling reaction started, proceeded to the reaction coil, RC, and formed a purplish-pink dye, giving peaks. The peaks were recorded in a PC connected to the FIA. Finally, the solution went to waste tank, W. The flow rates of the carrier, buffer and samples solutions were 2.6 mL min^{-1}. The concentration of nitrate-N was determined by measuring the peak area of the dye formed at 520 nm using the colorimetric detector. In case of non-injection of standard and samples, the carrier and buffer passed through a parallel channel, B, and not through C (Fig. 1). The FIA was operated at room temperature of 25 °C.

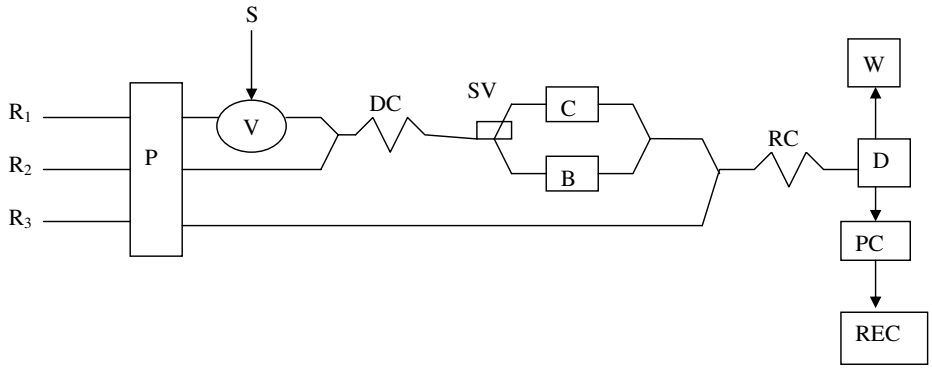

Fig. 1. Schematic diagram of the flow injection manifold used for the determination of nitrate-N: R_1, carrier stream (distilled de-ionized water); R_2, buffer stream (NH_4Cl/EDTA); R_3, color reagents stream (SA/NED); P, peristaltic pump; V, injection valve; S, sample; DC, delay coil; SV, two state switching valve; C, copperised cadmium column; RC, reaction coil; D, detector; W, waste tank; PC, personal computer; REC, recorder.

3. Results and discussion

Leafy vegetables are known to provide a significant portion of the nitrates in our diet. Approximately 5% of all dietary nitrates are reduced to nitrites in saliva and the gastrointestinal tract (Santamaria, 2006). Nitrites being highly unstable can be metabolized within the digestive tract to *N*-nitroso compounds (MAFF, 2001). *N*-nitroso compounds comprise of nitrosamines and nitrosamides (Ahn et al., 2002). Nitrosamines produced through acid catalysis of nitrite with certain nitrogen compounds are carcinogenic and volatile (Ezeagu, 1996; Perez-Olmos et al., 1997; Swann, 1975; White, 1975). Greater nitrite content thus could increase the likelihood of endogenous nitrosation reactions, which in turn may lead to a greater risk of cancer causation.

Suva is the capital city of Fiji. It has the highest urban population in the country. The Suva market is located next to the bus stand, which makes it an ideal and convenient place for consumers to purchase fresh vegetable produce. Thus four of the most commonly consumed leafy vegetables have been assayed in this study. The methodology employed to quantify nitrate-N is based on the Greiss protocol. Nitrite levels in fresh leafy vegetables are usually less than 2 mg kg^{-1} (Ximenes et al., 2000). Therefore, the contribution of nitrite towards the total nitrate load was not considered.

3.1. Calibration curves

The quantification of nitrate-N was carried out by employing an external calibration standard method. Fig. 2 shows typical flow signals, *i.e.*, peaks profiles for the calibration standards (1.0–20.0 mg L^{-1}) obtained through the FIA under optimum conditions and a representative calibration curve. The nitrate peaks obtained are clear and without interference from the organic matter in the vegetable matrices. The calibration graph was

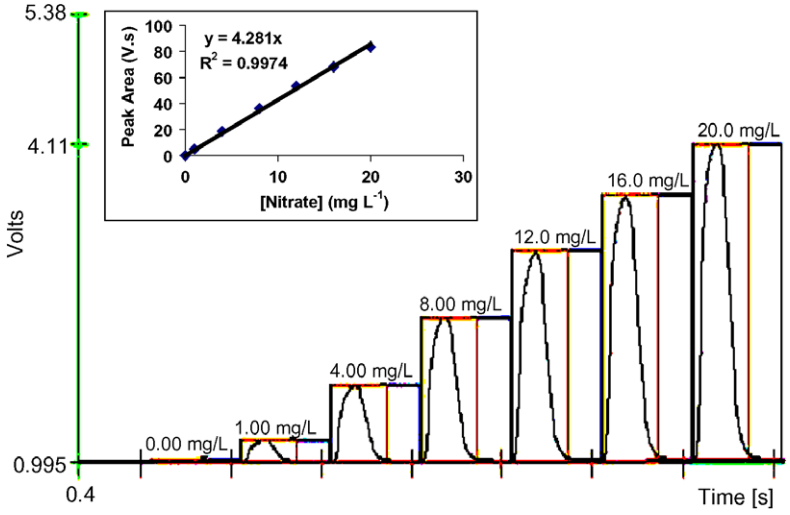

Fig. 2. Typical FIA peak profiles for standard nitrate-N (0–20 mg L^{-1}) and a representative calibration curve of measured peak area (V.s) *versus* nitrate-N concentration (mg L^{-1}).

Table 1
Assessment of extraction procedures: determination of nitrate-N (mg L^{-1}) extracted from fresh and spiked leafy vegetable samples

Sample	Activated carbon extraction			Alkaline extraction		
	Fresh samples	Spiked samples[a]	Recovery (%)	Fresh samples	Spiked samples[b]	Recovery (%)
Chinese cabbage	653.00 ± 2.83	775.00 ± 0.71	97.60 ± 2.83	222.00 ± 1.41	302.00 ± 2.83	100.00 ± 5.30
	758.00 ± 4.24	872.00 ± 4.24	91.20 ± 6.79	236.00 ± 0.71	323.00 ± 1.41	108.75 ± 1.77
	757.00 ± 1.41	896.00 ± 5.66	111.20 ± 3.39	204.00 ± 3.54	280.00 ± 2.83	95.00 ± 0.88
Celery	583.00 ± 2.12	696.00 ± 0.71	90.40 ± 2.26	196.00 ± 0.00	283.00 ± 4.95	108.75 ± 6.19
	480.00 ± 4.95	621.00 ± 16.26	112.80 ± 9.05	165.50 ± 0.71	246.00 ± 2.83	100.63 ± 2.65
	546.00 ± 7.78	675.00 ± 3.54	103.20 ± 3.39	177.00 ± 2.83	255.00 ± 0.00	97.50 ± 3.54
Lettuce	218.00 ± 3.54	358.00 ± 28.28	112.00 ± 19.80	66.20 ± 0.14	152.00 ± 1.41	107.25 ± 1.63
	188.00 ± 1.41	304.00 ± 2.12	92.80 ± 0.57	59.30 ± 0.00	143.00 ± 0.00	104.63 ± 0.00
	247.00 ± 0.71	365.00 ± 12.02	94.40 ± 9.05	80.40 ± 0.07	155.00 ± 6.36	93.25 ± 8.04
			Av. 100.62 ± 6.35			Av. 101.47 ± 6.29

[a] Each vegetable sample was spiked with 125 mg L^{-1} of nitrate-N and determined in duplicate.
[b] Each vegetable sample was spiked with 80 mg L^{-1} of nitrate-N and determined in duplicate.

obtained for each run by injecting six different concentrations of nitrate-N in the range 1.0–20.0 mg L^{-1} and plotting peak area (V.s) against concentration (mg L^{-1}). The calibration curves were linear in the concentration range studied with average correlation coefficient, $R^2 = 0.9974$. The calibration curves were used to calculate concentration of nitrate-N (mg L^{-1}) in selected vegetable samples and finally reported as mg kg^{-1}. Samples that exceeded the concentration range were diluted and reanalyzed.

3.2. Extraction and recovery

Two extraction techniques were assessed, *viz.*, activated carbon and an alkaline extraction using fresh and spiked vegetable samples, *i.e.*, Chinese cabbage, celery, and lettuce. A known amount of nitrate-N solution was added as an internal standard to all extracted samples. Table 1 shows a comparison of recovery data obtained from both extraction techniques for fresh and spiked samples. Under activated carbon and alkaline conditions average recoveries of nitrate-N for the three vegetables were 100.62 ± 6.35% and 101.47 ± 6.29%, respectively. A comparison of nitrate-N obtained from the two extraction techniques in fresh vegetable samples in mg kg^{-1} was also performed (not shown here). Both the extraction techniques gave almost similar results. However, activated carbon extraction, being less labor and time exhaustive, affords similar nitrate-N recovery in comparison to alkaline extraction and hence was the preferred extraction technique for all nitrate-N determinations in selected vegetables.

3.3. Method detection limit (MDL)

Method detection limit (MDL) was determined by analyzing the standard of known concentrations that fell between 0 ppm and the lowest calibration standard employed. The standard deviation, s, of seven replicates of this standard was acquired. The s was multiplied by the student's t (=3.14) value at the 99% confidence level to dispense the experimental MDL. The MDL for nitrate-N is 0.042 mg L^{-1} (0.34 mg kg^{-1}) which is sufficiently low for the determination of nitrate-N in selected leafy vegetable samples.

3.4. Method precision

Method precision was determined by analyzing results from an interday repeatability study. The relative standard deviation (RSD) of 10 repeated determinations of 2.70 mg L^{-1} standard solution of nitrate-N (Fluka Nitrate Ion Standard Solution no. 72544) was evaluated. The precision was established as acceptable with average stan-

Table 2
Nitrate-N content (mg kg^{-1}) in selected leafy vegetables before and after cooking (boiled, baked and fried)

Vegetable	Fresh[a]		Boiling			Baking			Frying		
	Mean	SD[b]	Mean	SD	Loss (−) (%)	Mean	SD	Gain (+) or loss (−) (%)	Mean	SD	Gain (+) (%)
English cabbage	1425.50	731.68	760.00	246.71	−46.69	1381.26	662.54	−3.10	3688.44	1566.82	+158.75
Lettuce	1297.14	347.85	653.04	246.28	−49.66	1320.04	324.71	+1.76	3626.40	795.74	+179.57
Celery	4706.74	1010.48	1922.99	387.06	−59.14	4783.82	994.08	+1.64	15063.06	4396.79	+220.03
Chinese cabbage	5658.08	738.70	2487.03	381.43	−56.04	5687.69	517.28	+0.52	23032.03	2170.80	+307.06

[a] Six samples ($n = 6$) for each type of vegetable were analyzed in duplicate.
[b] SD: standard deviation.

Table 3
A comparison of nitrate-N (mg kg^{-1}) contents in selected fresh leafy vegetables and the proposed method for the determination of nitrate-N with published methods and most of the FIA procedure along with the detection limits, dynamic range of detection (DRD) and other general characteristics of the methods

Vegetable	Year reported	Author	Country	Analytical method	Nitrate-N (mean)	Nitrate-N (range)	SD[a]	Detection limit (mg kg^{-1})	D.R.D[b] ($\mu g\ mL^{-1}$)	RSD[c] (%)	R_t[d] (min)	FIA sample throughput (h^{-1})
Cabbage	2007	Prasad and Chetty	Fiji	FIA with colorimetric detection 520nm	1425.5	724.4–2501.9	731.7	0.34	1.0–20.0	51.3	–	38, Present study
	2007	Thomson et al.	NZ	HPLC	275.0	100–575	166	5.0	–	60.4	–	–
	1998	MAFF-UK	UK	Anion-exchange HPLC - UV detection 210nm	318.0	83–655	–	7.5	–	–	–	–
	1993	Gundimeda et al.	India	Orion Ion-selective electrodes	380.0	–	130	–	–	34.2	–	–
Lettuce	2007	Prasad and Chetty	Fiji	FIA with colorimetric detection 520nm	1297.1	1010.7–1941.7	347.9	0.34	1.0–20.0	26.8	–	38, Present study
	2007	Thomson et al.	NZ	HPLC	1323.0	69–2846	874	5.0	–	66.1	–	–
	2003	Andrade et al.	Brazil	FIA with colorimetric detection 460nm	1880.0	–	181	20.0	1.0–10.0	9.6	–	30–40
	2001	MAFF-UK	UK	Anion-exchange HPLC - UV detection 210nm	1084.3	244–3073	–	–	–	–	–	–
	2000	van der Schee and Speek	The Netherlands	FIA with UV detection	2174[e]	80–4705	1124	50.0	–	51.7	–	–
	2000	Ximenes et al.	Brazil	UV Spectrophotometry 470nm	1945.0	–	–	39.0	–	1.0	–	–
				Polarography	1936.0	–	–	–	–	6.5	–	–
	1998	van der Schee	The Netherlands	FIA with UV detection	2100.0	–	–	50.0	–	–	–	–
	1998	MAFF-UK	UK	Anion-exchange HPLC - UV detection 210nm	1400.0	–	–	7.5	–	–	–	–
	1999	MAFF-UK	UK	Anion-exchange HPLC - UV detection 210nm	999.3	85–3857	–	7.5	–	–	–	–
	1991	Lyons et al.	Australia	Spectrophotometry	95.5	–	–	–	–	–	–	–
				HPLC-conductivity detection	79.0	–	–	0.12	–	–	–	–
	1985	Hunt and Seymore	UK	Anion-exchange HPLC - UV detection 210nm	5175.0	–	–	–	0.5–15 or 1.0–80	1.33	4.5	–
Celery	2007	Prasad and Chetty	Fiji	FIA with colorimetric detection 520nm	4706.7	3511.9–6008.1	1010.5	0.34	1.0–20.0	21.5	–	38, present study
	2007	Thomson et al.	NZ	HPLC	1339.0	732–1930	357	5.0	–	26.7	–	–
	1998	van der Schee	The Netherlands	FIA with UV detection	2900.0	–	–	50.0	–	–	–	–
	1991	Lyons et al.	Australia	Spectrophotometry	234.0	–	–	–	–	–	–	–
				HPLC-conductivity detection	326.0	–	–	0.12	–	–	–	–
				HPLC - UV detection	100.0	–	–	–	–	–	–	–
Chinese cabbage	2007	Prasad and Chetty	Fiji	FIA with colorimetric detection 520nm	5658.1	4523.8–6421.5	738.7	0.34	1.0–20.0	13.1	–	38, present study
	1998	van der Schee	The Netherlands	FIA with UV detection	3600.0	–	–	50.0	–	–	–	–
	1985	Hunt and Seymore	UK	Anion-exchange HPLC - UV detection 210nm	4848.0	–	–	–	0.5–15 or 1.0–80	0.99	4.5	–

[a] SD: Standard deviation.
[b] D.R.D: Dynamic range of detection.
[c] RSD: Relative standard deviation.
[d] R_t: Retention time.
[e] Total lettuce, summer + winter.

dard deviation and RSD values of 0.06 mg L^{-1} and 2.22%, respectively.

3.5. Determination of nitrate-N in fresh vegetables

The results of the investigation of the nitrate-N contents (mean values) of the selected fresh leafy vegetables are reported in Table 2 while their range and variability (RSD) are reported in Table 3. The mean nitrate-N content of the four leafy vegetables ranges from 1297 to 5658 mg kg^{-1}. Chinese cabbage has the highest nitrate-N content within the studied vegetables followed by celery, English cabbage and lettuce.

It is evident from the results shown in Tables 2 and 3 that there is considerable variation of nitrate-N content between different samples of the same vegetable. Table 3 clearly shows that vegetables with higher nitrate-N content tend to have lower variability between the different samples. The RSD ranges from 13.06% (Chinese cabbage) to 51.33% (English cabbage) (*cf.* Table 3). The high variability shown is satisfactory since vegetable nitrate-N content is known to vary extensively as reported in literature (van der Schee & Speek, 2000). Being partially a photosynthetic process in most plants, nitrate-N accumulation is understood to be independently reliant on the climate (Cardenas-Navarro, Adamowicz, & Robin, 1999). There is a general accord amid researchers that vegetables grown in low irradiance high temperature zones accumulate more nitrate-N (Cardenas-Navarro et al., 1999; McKnight, Duncan, Leifert, & Golden, 1999). In contrast to nitrate-N analysis of vegetables done in the UK (MAFF, 1998, 1999, 2001; van der Schee, 1998), except lettuce, the nitrate-N contents in Fiji's leafy vegetables could be considered slightly high but much higher in case of Chinese cabbage. Vegetable sampling was done during the months of 2006. From the meteorological data, *i.e.*, temperature, sunshine and rainfall (not shown here), for the Suva region during the sampling period 2006, it was evident that Fiji on average had both low and high irradiance with relatively high temperatures per day. Thus high nitrate-N values in part may be attributed to Fiji's tropical oceanic climate. Each vegetable sample was collected at random from six different vendors early in the morning. The amount of N-fertilizer and the amount of time after harvesting could not be ascertained. Variability between the samples may also be accredited to these two factors.

3.6. Determination of nitrate-N in cooked vegetables

Nitrate-N contents in fresh and cooked vegetables were determined using FIA and the mean data obtained are shown in Table 2 and compared in Fig. 3. Boiling reduces nitrate-N content since nitrate-N is soluble and predisposed to readily leach into the cooking liquids (Huarte-Mendicoa et al., 1997). The highest nitrate-N loss after boiling was found for celery (59.14%), followed by Chinese cabbage (56.04%), lettuce (49.66%) and English cabbage (46.69%). It is evident that the nitrate-N values are significantly reduced and similar observation is reported in literature (MAFF, 1999). The gain/loss data of nitrate-N after baking is also presented in Table 2, which indicates that the nitrate-N values remain relatively constant after baking. After studying the data regarding the nitrate-N values on frying in Soya bean oil, it may be categorically stated that there has been a 1.6–3-fold increase in nitrate-N content. The high nitrate-N gain may be attributed to the small sample size and the amount of oil used for frying. It is well established that the root nodules of legumous plants such as Soya possess special bacteria, which are able to fix elemental nitrogen and convert it to ammonia for the respective plant (Hill, 1996). Ammonia is later converted to nitrates through the nitrification process by other microor-

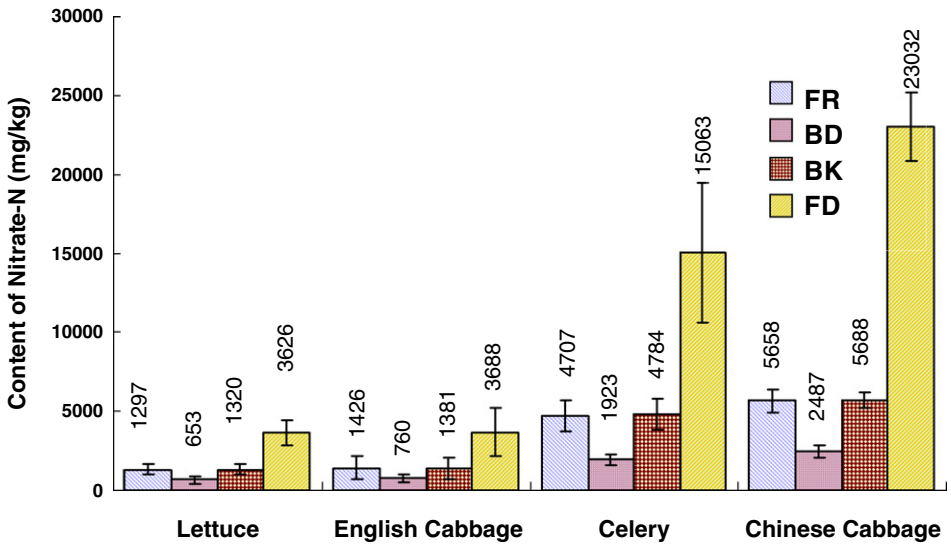

Fig. 3. Comparison of mean ($n = 6$) nitrate-N contents in fresh and cooked four leafy vegetables (FR – fresh, BD – boiled, BK – baked and FD – fried).

ganisms within the Soya plant. The prevalent nitrate-N increase through frying can be aligned to this fact but further detailed research is needed in this area.

3.7. Effect of deep-freezing

It has been shown that microbiological reduction of nitrates to nitrites takes place when foods are stored under refrigeration (Ezeagu, 1996). A similar case cannot be prevalent when foods are stored under deep-freezing since microbial action would not be expected to proceed at such low temperatures. In such cases where samples need to be stored frozen for a short period of time the effects of deep-freezing need to be examined. In this study the effects of deep-freezing on the nitrate-N content of the four selected vegetables were studied over a period of seven days. The nitrate-N content slightly fluctuated from the original, *i.e.*, fresh nitrate-N values over the seven day period. In terms of nitrate-N content a declining trend was evident although not anticipated in all the samples of four vegetables. The loss of nitrate-N over the seven day period in the vegetables studied were: Chinese cabbage 2.02%; celery 8.28%; lettuce 1.42% and English cabbage 10.94%. The minor loss of nitrate-N content may be attributed to any microbial action took place during the period when the samples are removed from deep-freezing and thawed.

3.8. Comparison of nitrate-N in fresh vegetables and analytical figure of merits

A comparison of nitrate-N contents ($mg\,kg^{-1}$) in selected Fiji's fresh leafy vegetables with the literature values available on similar leafy vegetables are presented in Table 3. A comparison of the proposed method for the determination of nitrate-N with other methods and most of the FIA procedures along with the detection limits, dynamic range of detection (DRD) and other general characteristics of the methods is shown in Table 3. From the analytical date presented in Table 3 it is clear that proposed FIA procedure for the determination of nitrate-N has much lower detection limit, higher dynamic range of detection and sample throughput.

4. Conclusion

Ever-increasing concern over nitrate ultimate toxicity has directed a number of countries to lay down maximum allowable threshold concentrations with regards to nitrate-N in vegetables (Lyons, McCallum, Osborne, & Nobbs, 1991). The nitrate-N content in four commonly consumed fresh leafy vegetables of Fiji has been determined. The effect of cooking and deep-freezing with regards to the four leafy vegetables has also been established. The nitrate-N values reported are slightly higher than those published in literature (MAFF, 1998, 1999, 2001; van der Schee, 1998) except lettuce where low value of nitrate-N was found (*cf.* Table 3). The FIA methodology described when employed with the activated carbon extraction technique, affords an accurate, rapid and precise measurement of nitrate-N in vegetables.

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References

Ahn, H. S., Yook, H. S., Rhee, M. S., Lee, C. H., Cho, Y. J., & Byun, M. W. (2002). Application of gamma irradiation on breakdown of hazardous volatile *N*-nitrosamines. *Journal of Food Science, 67*, 596–599.

Andrade, R., Viana, C. O., Guadagnin, S. G., Reyes, F. G. R., & Rath, S. (2003). A flow- injection spectrophotometric method for nitrate and nitrite determination through nitric oxide generation. *Food Chemistry, 50*, 597–602.

Cardenas-Navarro, R., Adamowicz, S., & Robin, P. (1999). Nitrate accumulation in plants: A role for water. *Journal of Experimental Botany, 50*, 613–624.

Cieslik, E., & Sikora, E. (1998). Correlation between the levels of nitrates and nitrites and the contents of potassium, calcium and magnesium in potato tubers. *Food Chemistry, 63*, 525–528.

Ezeagu, I. E. (1996). Nitrate and nitrite contents in ogi and the changes occurring during storage. *Food Chemistry, 56*, 77–79.

Gundimeda, U., Naidu, A. N., & Krishnaswamy, K. (1993). Dietary intake of nitrate in India. *Journal of Food Composition and Analysis, 6*, 242–249.

Higuchi, K., & Motomizu, S. (1999). Flow-injection spectrophotometric determination of nitrite and nitrate in biological samples. *Analytical Sciences, 15*, 511–518.

Hill, M. J. (1996). *Nitrates and nitrites in food and water*. England: Woodhead Publishing Limited.

Huarte-Mendicoa, J. C., Astiasaran, I., & Bello, J. (1997). Nitrate and nitrite levels in frozen broccoli. Effect of freezing and cooking. *Food Chemistry, 58*, 39–42.

Hunt, J., & Seymore, D. J. (1985). Method for measuring nitrate-nitrogen in vegetables using anion exchange high performance liquid chromatography. *Analyst, 110*, 131–133.

Kmiecik, W., Lisiewska, Z., & Slupski, J. (2004). Effects of freezing and storage of frozen products on the content of nitrate, nitrites, and oxalates in dill (*Anethum graveolens* L.). *Food Chemistry, 86*, 105–111.

Lima, J. L. F. C., Rangel, A. O. S. S., & Souto, M. R. S. (1995). Flow injection determination of nitrate in vegetables using a tubular potentiometric detector. *Journal of Agriculture and Food Chemistry, 43*, 704–707.

Lyons, D. J., McCallum, L. E., Osborne, W. J., & Nobbs, P. E. (1991). Assessment of procedures for the determination of nitrate and nitrite in vegetable extracts. *Analyst, 116*, 153–157.

MAFF: Ministry of Agriculture, Fisheries and Food. (1998). 1997/98 UK monitoring programme for nitrate in lettuce and spinach, food surveillance information sheet no. 154, August 1998, London.

MAFF: Ministry of Agriculture, Fisheries and Food. (1999). Nitrate in vegetables, food surveillance information sheet no. 158, September 1998, London.

MAFF: Ministry of Agriculture, Fisheries and Food. (2001). UK monitoring programme for nitrate in lettuce and spinach, food survey information sheet number 16/01, July 2001, London.

McKnight, G. M., Duncan, C. W., Leifert, C., & Golden, M. H. (1999). Dietary nitrate in man: Friend or foe? *British Journal of Nutrition, 81*, 349–358.

Mcmullen, S. E., Cassanova, J. A., Gross, L. K., & Schenck, F. J. (2005). Ion chromatographic determination of nitrate and nitrite in vegetable and fruit baby foods. *Journal of AOAC International, 88*, 1793–1796.

Monser, L., Sadok, S., Greenway, G. M., Shah, I., & Uglow, R. F. (2002). A simple simultaneous flow-injection spectrophotometric method based on phosphomolybdenum chemistry for nitrate and nitrite determinations in water and fish samples. *Talanta, 57*, 511–518.

Nagaraja, P., & Kumar, M. S. H. (2002). Spectrophotometric determination of nitrate in polluted water using a new coupling reagent. *Analytical Sciences, 18*, 355–357.

Oztekin, N., Nutku, M. S., & Erim, F. B. (2002). Simultaneous determination of nitrite and nitrate in meat products and vegetables by capillary electrophoresis. *Food Chemistry, 76*, 103–106.

Perez-Olmos, R., Herrero, R., Lima, J. L. F. C., & Montenegro, M. C. B. S. M. (1997). Sequential potentiometric determination of chloride and nitrate in meat products. *Food Chemistry, 59*, 305–311.

Reece, P., & Hird, H. (2000). Modification of the ion exchange HPLC procedure for the detection of nitrate and nitrite in dairy products. *Food Additive and Contaminants, 17*, 219–222.

Sanderson, J. E., Rassconsaul, J., & Lee, K. (1991). Nitrate analysis in meat. Comparison of two methods. *Journal of Food Science, 56*, 1123–1124.

Santamaria, P. (2006). Nitrate in vegetables: Toxicity, content, intake and EC regulations. *Journal of the Science of Food and Agriculture, 86*, 10–17.

Sen, N. P., & Donaldson, B. (1978). Approved colorimetric method for determining nitrate and nitrite in foods. *Journal of Association of Official Analytical Chemists (AOAC), 61*, 1389–1394.

Swann, P. F. (1975). The toxicology of nitrate, nitrite, and *N*-nitroso compounds. *Journal of the Science of Food and Agriculture, 26*, 1760–1770.

Tannenbaum, S. R., & Correa, P. (1985). Nitrate and gastric cancer risks. *Nature, 317*, 675–676.

Thomson, B. M., Nokes, C. J., & Cressey, P. (2007). Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water. *Food Additives and Contaminants, 24*, 113–121.

Tilbury, R. H. (1980). *Development in food preservatives-1*. London: Applied Science Publishers Ltd.

van der Schee, H. A. (1998). *The nitrate content of vegetables of the Dutch market in 1996*. Amsterdam: Inspectorate for Health Protection.

van der Schee, H. A., & Speek, A. J., (2000). Report of nitrate monitoring results concerning regulations EU 194/97. The Netherlands 2000, Amsterdam, Inspectorate for health protection and veterinary public health, Region Northwest.

White, J. W. Jr., (1975). Relative significance of dietary sources of nitrate and nitrite. *Journal of Agriculture and Food Chemistry, 23*, 886–891.

Wolff, I. A., & Wasserman, A. E. (1972). Nitrates, nitrites and nitrosamines. *Science, 177*, 15–18.

Ximenes, M. I. N., Rath, S., & Reyes, F. G. R. (2000). Polarographic determination of nitrate in vegetables. *Talanta, 51*, 49–56.